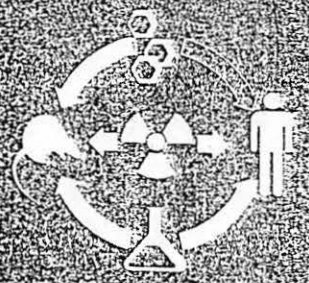
The Virus Cancer Program



June 1978

Division of Cancer Cause and Prevention

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

TABLE OF CONTENTS

A. VIRAL ONCOLOGY PROGRAM (VOP) - VIRUS CANCER PROGRAM (VCP)

1. Introduction

The Viral Oncology Program (VOP) is responsible for planning and conducting the Institute's program of coordinated research on the role of viruses in cancer. Scientists within the VOP conduct laboratory investigations and assist in the management of a collaborative program, the Virus Cancer Progra (VCP). These Programs have the following goals: (1) to search for viruses or virus genetic information related to the initiation of human cancer; (2) to elucidate the process by which normal cells become malignant using viruses as probes; and (3) to develop preventive and therapeutic measures for the control of human cancer.

Since its initiation the Virus Cancer Program has brought together investigators from around the world to focus their skills on the expansion of knowledge on the oncogenic properties of viruses and their relationship to human neoplasia. The Program has brought visibility to this problem area, resulting in an increased understanding of the nature of the interactions between viruses and cells. It has opened new vistas for biological investigation and has accelerated new developments far beyond its limited scope of activity. An awareness of the value of tumor viruses as tools for the study of biological processes now exists which had not been perceived when the Program was begun. We now know that different viruses can cause cancer in different mammalian species ranging from mice to large domestic animals and monkeys, and that such viruses may be transmitted through germ cells as well as from animal to animal. Highly specific probes have detected virus-related components in human cells. Techniques under development may improve diagnosis and prognosis for certain human cancers. The practical nature of much of this work was accomplished under this contract Program, first as the Special Virus-Leukemia Program, then the Special Virus-Cancer Program, and now the Virus Cancer Program.

The yearly budgets from the inception of the Program in 1964 to the present and the number of professional positions are given in Table 1.

2. Organization

a. Institute and Division Reorganization Relating to the VOP/VCP

During Fiscal Year 1978 the new Director of the National Cancer Institute, Dr. Arthur Upton, announced plans for a major reorganization of the Institut Three major changes are intended, encompassing the following:

- grant portfolios, and responsibility for their program management, wil be transferred from the Division of Cancer Research Resources and Centers (DCRRC) to other NCI divisions that manage similar or related programs; in most cases, relationships of grantees will continue with the same members of the NCI as at present;

- responsibility for all peer review activities, for both grants and contracts, will be transferred from other divisions to the DCRRC;

- DCRRC, which will continue to handle the administrative management of grants, will bear total responsibility for establishing policies under which NCI grants and contracts are to be used.

These improvements are designed to:

- bring about more effective integration of scientific and training activities of the NCI.

- bring the organization of NCI more nearly into confirmity with the organizational patterns of the other major NIH institutes;

- bring NCI into closer compliance with DHEW requirements for separation of program management from grant and contract administration, and from the peer review of grants and contracts; and

- give grant applications in all program areas an increased opportunity to compete on merit in the total dollar pool.

Actual implementation of the planned changes will take some time, and there will naturally be a period of transition and adjustment.

In October, 1977, Dr. Upton named Dr. Gregory T. O'Conor as Acting Director, DCCP, and in November, 1977, Dr. Louis R. Sibal (Deputy Associate Director, Viral Oncology Program) was asked to act as his Special Assistant. In January, 1978, Dr. J. B. Moloney, Associate Director, Viral Oncology Program, accepted a position in the Office of the Director, NCI. Shortly thereafter, Dr. Sibal was named Acting Associate Director, VOP.

In April, 1978, Dr. Upton appointed Dr. O'Conor to the permanent position of Director, DCCP. Dr. Sibal continues to serve as Acting Associate Director, VOP and as Special Assistant to Dr. O'Conor.

At the time of writing of this report, organizational changes were beginning to be implemented at Program, Division and Institute levels. A more detailed account of these changes will be presented in next year's annual report.

b. Viral Oncology Program (VOP)

The intramural and extramural programs of the Division will be administratively separated in the near future and coordinated by the Office of the Director, DCCP. At the time of this writing, however, the Viral Oncology Program (VOP) and the Virus Cancer Program (VCP) were organized as an integrated structure. The Office of the Associate Director, Viral Oncology Program, is presently responsible for the coordination of both the intramural and the collaborative research programs in cancer virology. The Program now has four Laboratories and one Branch. Each of these subdivisions has several sections, as listed in the Summary of Intramural Organization (Page 6). The term "laboratory" is used to describe the major intramural

subdivisions. The term "branch" refers exclusively to the extramural or collaborative program. The functional statements for each Office, Laboratory and Branch are given below; functional statements for Sections are found in the Laboratory reports. In general, the name of each Laboratory reflects its major research mission.

Functional Statements

Viral Oncology Program. (1) Plans, directs, coordinates and evaluates a program of basic and applied research on viruses as etiological agents of cancer; (2) establishes program priorities, allocates resources, integrates the projects of the various branches, evaluates program effectiveness, and represents program area in management and scientific decision-making meetings within the Institute; (3) administers research in biochemistry, tumors, genetics, pathology, biohazards, immunology, the environment, and viral and cell biology through intramural laboratories and contracts; (4) advises the Director of the Division and supports the activities of the National Cancer Advisory Board and other scientific advisory committees.

Office of Biohazard Safety. Performs research in virology, aerobiology, mammalian physiology, and biochemistry to evaluate the risk to the host when exposed to infectious agents. Develops and recommends equipment and procedures for handling of potentially biohazardous materials and disseminates this information to the interested scientific community throughout the world.

Collaborative Research Branch. Participates in the planning, development, and scientific administration of a program of collaborative research conducted within the Virus Cancer Program on viruses as etiologic agents of cancer in man and on the control of tumor viruses and/or their induced diseases. Provides research resources and logistical support to the VOP/VCP.

Laboratory of RNA Tumor Viruses. Plans and conducts research on the role of endogenous RNA viruses in natural and induced cancers of animals and man. Develops and applies methods for the prevention and control of cancers by vaccination with RNA viruses and by treatment with inhibitory host cell factors. Studies mechanisms of tumor suppression by chemotherapeutic and immunotherapeutic agents.

Laboratory of DNA Tumor viruses. Plans and conducts research on DNA viruses to define their role in the development of cancers in animals and man. Develops and applies biological, biochemical, and immunological procedures to obtain evidence for virus genetic expression in neoplastic cells. Conducts investigations to determine the mechanisms by which cellular gene expression, viral gene expression, and interactions between viruses influence transformation of cells.

Laboratory of Tumor Virus Genetics. Plans and conducts research to determine the molecular basis for the etiology of cancers. Conducts studies using molecular techniques to detect viral genes responsible for oncogenesis and to prevent their action. Develops assays for detection and characterization of

gene products involved in cellular transformation to malignancy. Elucidates the mechanisms by which the host regulates the expression of oncogenic viruses.

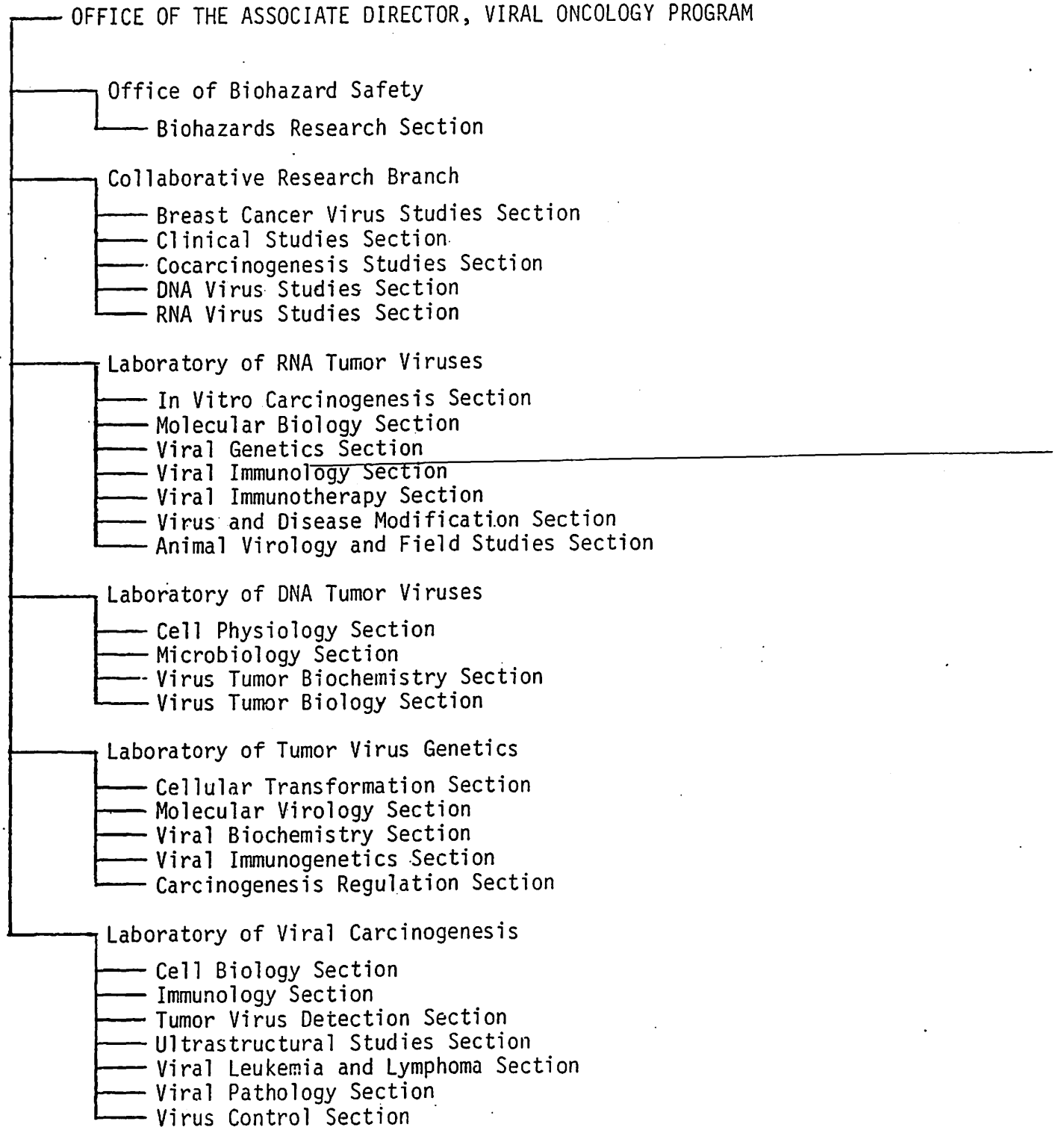
Laboratory of Viral Carcinogenesis. Plans and conducts research on virus-host relationships in virus-induced cancers with emphasis on the detection and characterization of oncogenic viruses and the mode of viral transmission in animals and man. Studies the interactions of viral and cellular genes. Studies host immune mechanisms related to the control of virus-induced cancers. Conducts investigations on molecular processes in viral carcinogenesis.

Table 1

FUNDING HISTORY OF THE VIRAL ONCOLOGY PROGRAM
(in thousands)

Fiscal Year	Number of Positions	In-House	VO Contracts	SVLP	VCP	BCTF	CREG	TOTALS
1964	30	—	4926	—	—	—	—	
1965	117	1687	5433	8723	—	—	—	15,843
1966	140	1835	3064	13,556	—	115	—	18,570
1967	144	1999	3137	13,505	—	246	—	18,887
1968	157	2239	—	—	17,241	284	—	19,764
1969	176	2891	—	—	17,985	259	—	21,135
1970	180	3356	—	—	17,340	174	—	20,870
1971	197	4517	—	—	31,591	234	—	36,342
1972	226	6310	—	—	41,889	734	—	48,933
1973	219	6983	—	—	42,564	1006	—	50,553
1974	231	7189	—	—	49,553	1150	—	57,892
1975	229	9395	—	—	49,387	1450	—	60,232
1976	222	10,800	—	—	46,773	1450	967	59,990
1977	234	12,547	—	—	44,450	1450	1800	60,247
1978	234	15,296	—	—	41,171	1450	1800	59,717

Summary of Intramural Organization



Intramural Review Committees

Viral Oncology Program Coordinating Committee

Dr. Stuart Aaronson
Dr. James Duff, Acting Chairman
Dr. Peter Fischinger
Dr. Berge Hampar
Dr. David Howell, Executive Secretary
Dr. Robert Huebner
Dr. Robert Manaker
Dr. Jeffrey Schlom
Dr. Edward Scolnick
Dr. Louis Sibal
Dr. George Todaro
Dr. George Vande Woude

Viral Oncology Program Resources & Logistics Advisory Group

Dr. Jack Gruber, Chairman
Dr. Garrett Keefer, Executive Secretary
Dr. Clarice Gaylord
Dr. Maurice Guss
Dr. Takis Papas
Dr. Ernest Plata
Dr. John Stephenson
Dr. David Troxler
Dr. Daniel Twardzik
Dr. George Vande Woude
Dr. Wilna Woods

Clinical Advisory Group for Viral Oncology

Dr. Michael Blaese (DCBD)
Dr. Peter Fischinger (DCCP)
Dr. Robert Gallo (DCT)
Dr. Sylvan Green (DCCP)
Dr. Curtis Harris (DCCP)
Dr. Ronald Herberman (DCBD)
Dr. Paul Levine (DCCP) - Chairman
Dr. Franco Muggia (DCT)
Dr. Alan Rabson (DCBD)

Contract Specialists (Research Contracts Branch)

* Mr. William Caulfield
Mr. James Doyle
** Mr. Charles Fafard
Mr. Sidney Jones
Mr. Jacque Labovitz
Mr. J. Thomas Lewin
Mr. Thomas Porter
Mr. Clyde Williams

---

* Deputy Chief
** Chief

Virus Cancer Program Scientific Review Committee

Dr. David Bishop, University of Alabama in Birmingham (Birmingham, AL)
* Dr. Paul Black, Massachusetts General Hospital (Boston, MA)
Dr. George Blumenschein, University of Texas Systems Cancer Ctr (Houston, TX
Dr. Ronald Glaser, Ohio State University (Columbus, OH)
Dr. Peter Gomatos, Sloan-Kettering Institute (New York, NY)
Dr. Adeline Hackett, Peralta Cancer Research Institute (Oakland, CA)
Dr. Jacob Holper, Litton Bionetics, Inc. (Kensington, MD)
Dr. Albert Kaplan, Vanderbilt University (Nashville, TN)
Dr. Miriam Lieberman, Stanford University (Stanford, CA)
Dr. Frank Lilly, Albert Einstein College of Medicine (Bronx, NY)
Dr. Diana Lopez, University of Miami (Miami, FL)
Dr. Robert McAllister, Children's Hospital of Los Angeles (Los Angeles, CA)
Dr. Daniel Medina, Baylor College of Medicine (Houston, TX)
Dr. Garth Nicolson, University of California (Irvine, CA)
Dr. Gary Pearson, Mayo Clinic (Rochester, MN)
Dr. Malcolm Pike, USC School of Medicine (Los Angeles, CA)
Dr. William Rawls, McMaster University (Hamilton, Ontario, CANADA)
Dr. Marvin Rich, Michigan Cancer Foundation (Detroit, MI)
Dr. Charles Rickard, Cornell University (Ithaca, NY)
Dr. Phillips Robbins, Massachusetts Institute of Technology (Cambridge, MA)
Dr. William Summers, Yale University School of Medicine (New Haven, CT)
Dr. Arthur Weissbach, Roche Institute of Molecular Biology (Nutley, NJ)
Dr. Adel Yunis, University of Miami (Miami, FL)

Dr. Clarice Gaylord, NCI, NIH (Executive Secretary)
Dr. Maurice Guss, NCI, NIH (Executive Secretary)
Dr. Wilna Woods, NCI, NIH (Executive Secretary)

---

* Chairman

CONSULTANTS (October 1, 1977 - September 30, 1978)

Dr. Ervin Adams, Baylor College of Medicine (Houston, TX)
Dr. Ralph Arlinghaus, University of Texas (Houston, TX)
Dr. Carlo Croce, Wistar Institute (Philadelphia, PA)
Dr. Etienne de Harven, Memorian Sloan-Kettering Cancer Center (New York, NY)
Dr. Erwin Fleissner, Sloan-Kettering Institute for Cancer Research (New York, NY)
Dr. Murray Gardner, University of Southern California (Los Angeles, CA)
Dr. Peter Gerone, Delta Regional Primate Center (Covington, LA)
Dr. Raymond Gilden, Litton Bionetics, Inc. (Frederick, MD)
Dr. Anthony Girardi, Institute for Medical Research (Camden, NJ)
Dr. Maurice Green, St. Louis University (St. Louis, MO)
Dr. Thomas Hakala, University of Pittsburgh (Pittsburgh, PA)
Dr. Elizer Hueberman, Oak Ridge National Laboratory (Oak Ridge, TN)
Dr. Herbert Lazarus, Sidney Farber Cancer Center (Boston, MA)
Dr. Richard Lerner, Scripps Clinic and Research Foundation (La Jolla, CA)
Dr. Harvey Rabin, Litton Bionetics, Inc. (Frederick, MD)
Dr. Richard Steeves, Albert Einstein College of Medicine (Bronx, NY)
Dr. Robert Weinberg, Massachusetts Institute of Technology (Cambridge, MA)
Dr. Lauren Wolfe, Rush-Presbyterian-St. Luke's Medical Center (Chicago, IL)

c. The Virus Cancer Program (VCP)

Background. At the present time the Virus Cancer Program is administered by the Collaborative Research Branch with five Sections: the Breast Cancer Virus Studies Section; the Clinical Studies Section; the Cocarcinogenesis Studies Section; the DNA Virus Studies Section; and the RNA Virus Studies Section. Virus-cancer contracts are managed by the heads of each Section under the general supervision of the Branch Chief, the Associate Chief, and the Office of the Associate Director; Section heads do not conduct intramural research.

The VCP Advisory Committee, formerly charged with responsibility for providing advice on overall Program direction, was dissolved during FY 1978 at the direction of the Secretary, DHEW, as part of an effort to reduce the number and size of standing committees in the Department. VCP Scientific Review Committee B was also abolished as a result of the same effort; its functions were transferred to the VCP Scientific Committee, a streamlined continuation of the former VCP Scientific Review Committee A. It is anticipated that this committee may be merged with the Carcinogenesis Scientific Review Committee to form the Cause and Prevention Review Committee.

Review of Contracts. The following is an outline of the methods used until very recently for the review of research contracts. It is included in this report since many of these procedures were followed until October 1, 1978, when the reorganization of the Division was to be completed. At that time, new procedures were to be instituted. It should also be pointed out that contracts presently engaged in basic research are expected gradually to be phased out in coming years and their support transferred to the grants area of the DCCP if appropriate.

Projects within the total Program are reviewed at many levels:

(1) Each contract is reviewed for relevance, priority and need to the total Program by the Virus Cancer Program Coordinating Committee (VCPCC). This committee, composed of members of the Office of the Associate Director, the Collaborative Research Branch, and senior intramural investigators, reviews solicited and unsolicited proposals received by the Program. Consensus is obtained by open vote and priority is established by scoring on secret ballot.

(2) Each contract is reviewed for scientific excellence and technical competence by the Scientific Review Committee. This committee, composed entirely of non-NCI scientists, operates entirely under the regulations set forth by the OMB, DHEW, NIH, and NCI, with Executive Secretaries who are responsible for the review and complete documentation of each project.

(3) Each contract is continually monitored for performance by (a) the Collaborative Research Branch; (b) CRB Section Heads; (c) Project Officer(s). Project Officers are intramural senior laboratory investigators who serve as advisers to principal investigators on scientific matters.

(4) Each contract above the annual funding level of $1 million and with multifaceted workscope undergoes a third review by an ad hoc committee appointed by the Associate Director, Viral Oncology Program.

(5) Each contract is reviewed by the Associate Director, Viral Oncology Program; the Director, Division of Cancer Cause and Prevention; the Chief, Research Contracts Branch, NCI; and, as required, by the Director, NCI.

As an aid to the review processes, key staff members receive progress reports on all contracts on a biannual basis. Collection and distribution of these reports is the responsibility of the Editorial Unit in the Office of the Associate Director. A single comprehensive report is prepared annually by the Associate Director.

Flow Plan for Solicited Proposals.

(1) Project idea conceived and developed by program.

(2) Coordinating Committee review. (Originator of plan presents to Committee for determination of relevance, priority, and need.)

(3) Project Plan document and RFP drafted. (Originator and Contract Specialist prepare the Project Plan and the RFP.)

(4) Technical review and contractor selection. (Competitive processes completed as required. Scientific Review Committee reviews technical aspects and ranks competing proposals. Program staff selects contractor(s)).

(5) Project Plan document approved and Review Summary Sheet prepared and approved. (Project Officer and Contract Specialist prepare Review Summary Sheet and route Project Plan and Review Summary Sheet for signatures.)

(6) Negotiation and award. (Contract Specialist negotiates contract with help of Project Officer as required. Preliminary discussions and negotiations may have occurred under Contractor Selection and Technical Review.)

Flow Plan for Unsolicited Proposals.

(1) Unsolicited proposal received by Program.

(2) Coordinating Committee review. (Coordinating Committee determines relevance, priority, need and uniqueness.)

(3) Project Plan drafted. (Project Plan document, Justification for Non-Competitive Procurement, prepared by Program staff member and Contract Specialist.)

(4) Technical review. (Technical review of proposal performed by Scientific Review Committee.)

(5) Project Plan and Review Summary Sheet approved. (Contract Specialist routes Project Plan document and Review Summary Sheet for signatures.)

(6) Negotiation and award. (Contract Specialist negotiates contract with help of Project Officer as required.)

Scientific Staff - Office of the Associate Director, Viral Oncology Program

Dr. Louis R. Sibal, Acting Associate Director
Dr. David M. Howell, Assistant to the Acting Associate Director
Dr. Henry J. Hearn, Scientific Coordinator for Viral Oncology, Frederick Cancer Research Center (FCRC)

Administrative Staff - Office of the Associate Director, Viral Oncology Program

Mr. John Miller, Administrative Officer, DCCP
Mr. Nicholas Olimpio, Program Analyst, DCCP
Mr. Robert Velthuis, Administrative Officer, VOP
Mr. Roy Hendricks, Assistant Administrative Officer, VOP

3. Scientific Activities

a. Narrative

Introduction

The conquest of cancer has been described as the most complex problem yet to challenge biomedical science. It is therefore not surprising that the course of research toward solution of the problem has taken unexpected turns and that, as new knowledge has become available, old ideas have been modified or abandoned entirely.

Research on the involvement of viruses in cancer is no exception. The initial skepticism that greeted the discoveries of the early tumor virologists gradually gave way to acceptance of the fact that certain viruses predictably and reproducibly cause malignancy in animals. The ensuing isolation of viruses responsible for cancer in avian, amphibian, murine, feline, bovine and primate species led logically to the search for similar agents in humans. In the course of this search, however, it has become apparent that the viruses involved in human cancer are not necessarily to be thought of as discrete particles which cause malignancy in the same predictable fashion as the tumor viruses of some lower animals. Instead, it is increasingly likely that viral information, present as nucleic acid sequences in the genes of apparently normal human cells and replicating with them, is intimately involved with the development of cancer. Triggered by chemical carcinogens, radiation, hormones, the aging process, and other influences, these virus sequences may direct the synthesis of the proteins responsible for malignant transformation of the cell. Indeed, where appropriate detection systems have been developed, the carcinogenic activity of chemical and physical agents has often been found to be accompanied by the activation of a latent viral genome.

Several exciting developments have recently occurred which emphasize not only the central role that viruses and viral information may play in human cancer but also underscore the enormous value of viral oncology research in understanding the basic mechanisms by which the disease is induced. The isolation of proteins responsible for malignant transformation and the identification of the viral genetic sequences that code for them place at the disposal of the research scientist reproducible means by which oncogenic mechanisms can be scrutinized. Moreover, the discovery that some of the most potent oncogenic viruses derive the information coding for their transforming proteins by recombination of viral/viral or viral/cellular genetic sequences now allows a rigorous examination of those factors regulating the genesis of oncogenic biological materials.

RNA Viruses

The RNA viruses are now generally divided into three morphologically distinct groups: type C viruses, associated with leukemias, sarcomas and lymphomas; type B viruses, associated with carcinoma of the breast; and the recently classified type D viruses, whose functions are presently being analyzed.

These agents, first to be identified as causing malignancy in animals, are unique among animal viruses in their mode of transmission and the intimate association that has evolved between them and the cells of a wide variety of vertebrates. Perhaps the most striking advances in understanding the molecular mechanisms of cancer have been made using RNA tumor viruses in animal model systems, and it has been a strong point of the Program that the knowledge and technology resulting from these advances are immediately focused on the attempt to discover the processes by which cancer occurs in humans.

Type C Viruses. The etiologic role of this group of viruses has been established for naturally occurring cancers of many species, including some non-human primates. Certain members of the group, the so-called "replication-defective" transforming viruses, appear to have arisen by a mechanism involving recombination with cellular transforming genes. As such, these viruses offer an unparalleled opportunity to elucidate the processes by which genetic information can, under the proper circumstances, cause malignancy.

Two of these agents, Kirsten murine sarcoma virus (KiSV) and Harvey murine sarcoma virus (HaSV), each capable of transforming undifferentiated fibroblasts in culture, have been shown to be recombinants between mouse and rat type C viruses. Their genomes do not appear to code for any of the structural proteins of known mammalian retroviruses; instead, two new proteins have been found which are coded for by these agents: a 20,000 dalton protein specified by the HaSV genome and a 50,000 dalton protein specified by the KiSV genome. The origin of the p22 is presently unclear, but evidence has been accumulated that the 50,000 dalton protein is coded for by the KiSV genetic sequences which were derived from the rat. This is important because the p50 also seems to be synthesized by these sequences when they are expressed in naturally occurring rat tumors. In addition, KiSV-related rat sequences are expressed at high levels in carcinogen (DMBA)-induced rat mammary tumors, and these sequences include that portion of the viral genome that codes for the KiSV-specific p50. Assays for this protein should therefore be useful in the diagnostic evaluation of chemical carcinogen-induced tumors in rats and can provide new, sensitive and rapid assays for chemical carcinogens in this widely used rodent testing system.

The protein responsible for the highly leukemogenic potential of Friend spleen focus-forming virus (SFFV) has been recently identified. A glycoprotein with a molecular weight of 60-70,000 daltons, this transforming molecule is coded for by sequences which SFFV acquires through recombination. It is located at the cell surface and a radioimmunoassay has been developed which detects its presence. The isolation of this protein is a hallmark in leukemogenesis research because it allows for the first time the identification of a protein responsible for the conversion of a normal, hematopoietic precursor to a leukemic cell. Since the molecule is a glycoprotein and situated on the surface of the cell, it presents a number of exciting possibilities for immunologic prevention and therapy of leukemia. In addition, a protein with a molecular weight of 50,000 daltons has been found to be the gene product responsible for the transforming activity of Rous sarcoma virus. This

important discovery assumes additional significance because the protein, like the transforming glycoprotein of SFFV, is the product of recombinant genetic sequences. Purification and analysis of these transforming molecules will offer critical insight into the ways RNA tumor viruses cause cancer.

Analysis of the translational products of replication-defective transforming viruses has continued in feline systems as well. The helper virus proteins expressed in cells transformed by feline sarcoma virus, itself a defective agent, have been shown to be translated initially in the form of a high molecular weight precursor. This precursor contains the antigenic reactivity of the feline oncornavirus-associated membrane antigen (FOCMA), which has been found in naturally occurring feline leukemias and sarcomas whether they produce virus or not. If FOCMA is therefore a product of the FeSV genome, it should be feasible to purify and further characterize this protein. This is important because antibody to FOCMA seems to be predictive of resistance to the development of feline lymphoma, and isolation of the antigen could therefore be of great benefit in testing the immunoprevention of FeLV/FeSV-induced tumors. Moreover, since FOCMA appears to be a virus-directed protein, availability of the antigen would be of great importance in determining its role in transformation.

New viruses resulting from genetic recombination between exogenous and endogenous type C mouse viruses have been identified by analysis of their gene products. This technique has also been used to investigate genetic relationships between different endogenous viruses that exist within the same mouse cells. The results of this research appear to indicate that the inducible, xenotropic virus of mouse cells arises by recombination between two other endogenous viruses. This phenomenon can be useful because recombination between endogenous viral genes provides a mechanism for gene amplification in eukaryotic cells. In addition, by appropriate immunologic and genetic analysis of the recombinant viruses resulting from crosses between leukemogenic and nonleukemogenic mouse viruses, it may now be possible to identify and localize a region of the replication-competent type C virus genome that is responsible for malignant transformation of lymphoid cells *in vivo*.

In other studies, the RNAs of replication-defective murine and primate transforming viruses were analyzed for the presence of nucleotide sequences homologous to the genomes of their respective helper type C viruses. The defective viruses varied widely in their ability to express helper virus *gag* proteins and in their total conservation of helper virus sequences. In striking contrast, 5' terminal sequences of the helper virus were found to be conserved in the RNA of every sarcoma virus tested. These findings indicate that a general property of mammalian transforming type C viruses is their conservation of helper virus 5' terminal nucleotide sequences and suggests a critical role for these sequences in the life cycle of the defective transforming virus. The continued application of molecular, biological and immunological techniques to the study of mammalian transforming viruses is expected to yield critical information regarding the organization of their genomes and the mechanisms by which their translational products induce striking and rapid transformation of cells in culture and *in vivo*.

Recent research has shown that as much as 0.1% of the genome of rodent and primate cells is composed of sequences homologous to murine and primate retroviruses, respectively. In some cases, sequences homologous to the genes of RNA tumor viruses occupy as high a percentage of the cell's genome as does the cell's ribosomal RNA. This discovery indicates that these sequences play some role in the function of the cell which has been conserved in evolution, since they are subjected to the same evolutionary pressures as are cellular DNA sequences. Endogenous retroviruses may thus be regarded as basically cellular genes, present in multiple copies, which also have the ability to give rise to infectious, potentially pathogenic virus particles.

Cellular control of potentially oncogenic viral sequences is obviously of critical importance in determining the susceptibility of the cell to malignant transformation. Because of this, the mammalian chromosomal genes which are related to neoplastic transformation have received increasingly intensive scrutiny. Among these are the mouse genes Fv-1 and Fv-2, which control the development of murine leukemia; RGV-1, RFV-1, and RRV-1, which confer resistance to Gross, Friend, and radiation leukemia viruses, respectively; Ram-1 and Rec-1, which specify receptor sites for amphotropic or ecotropic MuLV.

Of even greater potential value, however, is the fact that human genes are now being identified which may be involved in transformation. The Bevi (baboon endogenous viral integration) gene, assigned to human chromosome six, has been shown to be the integration site of baboon endogenous virus in human cells. The EGF gene is thought to control cellular receptor sites for epidermal growth factor, while the NGF gene controls receptors for nerve factor; these sequences are important because the uncontrolled growth of malignant cells is related to the altered activity of certain hormone-like substances with the cell surface. The interaction of receptor sites with specific growth factors causes an overstimulation of cell division which results in the abnormal multiplication seen in cancer.

It has been suggested that chemical carcinogens may act in concert with viral information already in the cell. This idea is supported by the finding that induction of certain tumors by chemical carcinogens results in the concomitant expression of antigens of endogenous type C viruses; this is turn is linked with rapid cell proliferation. Chemically-induced primary tumors in rats expressed only low levels of viral antigens, but secondary transplants and subsequent passages progressively elevated the level of viral antigens as the tumors grew more rapidly and metastasized. This is significant because it could mean that definable viral marker proteins may be useful as indicators of secondary tumor growth and progressive de-differentiation of tumor cells.

Some chemically-transformed mouse cell lines have been found to produce the same DNA-binding regulatory proteins as other lines transformed by DNA tumor viruses. These proteins are not found in normal cells. Two independently isolated cell lines, one transformed by DMBA and the other by benzpyrene, make new DNA-binding proteins which appear to be closely related, if not identical, to one another. This suggests that different chemical carcinogens may affect common growth regulatory systems within target cells and may act like DNA tumor viruses in inducing new proteins which bind selectively to cellular DNA.

Immunoprevention of virus-induced cancer continues to receive increasing attention. Protection against leukemia in AKR mice and highly significant prevention of 3-methylcholanthrene-induced sarcomas in mice have been accomplished with passive immunization. However, only those antisera which possessed high titers against both ecotropic and xenotropic viruses offered significant protection, while sera with relatively low titers against xenotropic viruses offered little protection. This suggests that antibodies against specific antigenic determinants may be found which will be important in immunoprevention of malignancy in this system.

Both syngeneic and allogeneic KiSV-induced tumor cell vaccines were shown to provide transplantation immunity to a variety of lethal, syngeneic rat tumors which were originally induced by chemicals, DNA tumor viruses, and the aging process. While these results further underscore the concept that KiSV translational products are involved in the development of murine cancer, they also suggest means for its control and ways by which the mechanism of transformation can be elucidated.

Type B Viruses. Although breast cancer occurs in many different mammals, the mouse is the only species in which a viral etiology for the disease has been definitely established. The causative agent, mouse mammary tumor virus (MMTV), has been carefully scrutinized from the viewpoints of genetics and molecular biology. Genetic analysis of host control has shown that at least three mouse genes appear to influence MMTV tumorigenesis. A single dominant gene, Mtv-1, controls the release of a mildly oncogenic variant of MMTV in C3Hf mice, while a second gene, Mtv-2, determines expression of a highly oncogenic strain of MMTV in GR mice. It is believed that the third, a repressor gene, inhibits the release of MMTV provirus which is integrated into the genome of most, if not all, strains of mice.

As the loci responsible for expression of endogenous genetic variants of MMTV are identified, congenic strains of mice have been produced with specific genetic deletions which permit the performance of linkage studies for gene mapping. A congenic strain of mice, GR/MTV-2, has recently been established which lacks the Mtv-2 locus, does not shed MMTV in milk, and does not develop early mammary tumors. This is important because these mice can be used as an essential control in studying the oncogenic action of the Mtv-2 gene.

Significant progress has been made in the elucidation of the structure, assembly, and maturation of MMTV. It has been shown that the glycoproteins gp52 and gp36 are located on the surface of the viral envelope, while the non-glycosylated polypeptides p28 and p14 are core proteins and p10 is membrane-associated. It has been suggested that during maturation it is the p10 which links the nucleocapsid precursor of MMTV to the envelope. Non-murine cells have been productively infected with several strains of MMTV showing high oncogenicity. Newly synthesized MMTV can be detected as early as four days after infection by these strains, thereby providing a basis for a rapid in vitro assay of the presence of infectious MMTV. This is also important because the system affords the means by which the potentially co-carcinogenic effects of MMTV and physical or chemical agents can be studied.

MMTV proviral sequences have been detected in the cellular DNA of livers and mammary tumors in RIII and C3H mice. However, the mammary tumors carry more of these sequences than liver tissue, and it has been learned that some of the sequences are apparently unique to the tumors. It therefore seems likely that there are certain sequences, present as proviruses in the mammary tumor DNA of mice having a high incidence of breast cancer but absent from the DNA of moderate and low incidence strains, that are transmitted by some mechanism other than through the germ line. Of related interest is the fact that small differences have been found to exist between nucleic acid sequences of the highly oncogenic MMTVs.

Type D Viruses. The first virus of this group to be isolated was the Mason-Pfizer monkey virus (MPMV). During the past year, similar viruses, among which are langur virus (LV) and squirrel monkey retrovirus (SMRV), have been isolated from New and Old World primates.

Analysis of these important agents has yielded an interesting pattern of similarities and differences. For example, MPMV and LV appear to be remarkably similar with respect to the electrophoretic inabilities of their major polypeptides, while the polypeptide pattern of SMRV is quite distinct from the other two. However, radioimmunoassays for these polypeptides show distinct differences between all three agents, particularly with respect to the low molecular weight polypeptides. In addition, the major structural protein (p35) of SMRV has antigenic determinants which are distinct from the proteins of all other known viruses. On the other hand, in more broadly reactive immunoassays, SMRV p35 was shown to cross-react with the major structural protein (p26) of MPMV.

These findings are important for several reasons. These agents are endogenous viruses from widely separated species and their similarities suggest that a progenitor ot the type D viruses became genetically associated with primates early in their evolution. Conservation of common sequences under evolutionary pressure raises the possibility that they may serve some necessary function under normal circumstances. Equally significant, however, is the fact that RIA's have been established for the major polypeptides of these viruses which now make it possible to search for their presence in human tumor cells.

New Viruses. Several new endogenous type C viruses have been isolated and characterized within the past year. One of these, a B-tropic virus inducible from the SWR inbred mouse strain, was shown to be distinguishable by molecular hybridization from previous endogenous mouse type C virus isolates. Nucleotide sequences specific to this virus were found only within cellular DNA of SWR mice and not in other inbred mouse strains. In addition, the inducibility locus for the SWR virus was shown to segregate with the viral structural information in genetic crosses, indicating that this virus is transmitted in the host in a Mendalian manner.

A new, endogenous type C virus of carnivores has also been isolated and characterized. Cells of the established MvlLu mink line were found to spontaneously release a reverse transcriptase-containing virus after long term passage in tissue culture. By molecular hybridization, DNA of normal mink cells was found to possess extensive nucleotide sequence homology with a DNA copy of the viral genome. The new agent, called mink endogenous virus, shares several antigenic determinants with the major structural proteins of known mammalian type C viruses, while possessing other determinants unique from those of other known retroviruses. By immunologic criteria, it most closely resembles FeLV and the endogenous type C virus of the rat.

DNA Viruses

The role of DNA viruses as etiologic agents of proliferative cellular diseases in several animal species is now well accepted. Early reports describing the capacity of certain murine and simian papovaviruses to induce malignant tumors in animals and transform cells in tissue culture provided the impetus for examining the relationship between various human DNA viruses and cancer in man.

The first class of viruses considered in this context were the human adenoviruses which have been shown to act as biological carcinogens in selected rodent systems. Prior to this demonstration, various live, attenuated adenovirus vaccines were administered to selected human populations as a control measure for debilitating respiratory tract infections. A further complication was introduced when it was discovered that the oncogenic papovavirus SV40, acquired from the simian cells used for propagation of the adenoviruses, was present as a major contaminant in these vaccine preparations. Since hybrid viruses with a spectrum of biological functions have been isolated from mixed adenovirus-SV40 populations, these adenovirus vaccines undoubtedly contained such recombinant viruses. Thus, more than one million people were inoculated with representative members of two groups of DNA viruses with known oncogenic properties.

The possibility that members of this group might be involved in human cancer was investigated by screening sera from cancer patients for antibody to the T-antigens induced by these viruses in transformed cells. No significant patterns of serological reactivity emerged from such studies. Hundreds of human tumor specimens were then examined for adenovirus-specific mRNA and for viral DNA using probes that could detect each of 31 types of adenovirus, but again no evidence of viral information was found in these specimens. The continued study of a possible association between these viruses and human cancer still seems relevant because: (1) these agents produce malignant tumors in several species of animals and morphologically transform a variety of cultured cells, including those of human origin; (2) human adenoviruses are widely distributed and interact genetically with the oncogenic simian virus, SV40, inadvertently administered to a large group of people as a vaccine contaminant; (3) several human papovaviruses have been found to be widely distributed in human populations; (4) genetic information homologous

to one of these agents (BK virus) has been detected in several human tumors; and (5) these small DNA viruses offer useful models for studying the mechanisms underlying the process of cell transformation.

The papovaviruses have provided a particularly fruitful approach for studying the phenomenon of transformation of a cell from the normal to the malignant state. These small, relatively simple viruses perform the convenient function of packaging all of the genetic information required for the induction of cancer. The fact that the DNA of these viruses codes for the synthesis of less than 10 proteins makes them especially attractive in terms of defining those virus-specific functions which are responsible for oncogenesis. Indeed, significant progress has already been made along these lines. Cleavage of the viral DNA with restriction endonucleases has led to the generation of probes representative of specific portions of the viral genome. These probes have been used to identify the transforming genetic sequences of SV40 and certain adenoviruses and to map the cellular integration sites of SV40 in transformed cells. Analysis of cells transformed by mutants of SV40 has led to the identification of a virus-coded protein (A protein) which is required to initiate and maintain cell transformation. This early viral protein, related immunologically to the intranuclear tumor antigen (T antigen) found in all SV40-transformed cells, is also required for the initiation of viral DNA synthesis. The T antigen has now been extracted from transformed cells and experiments have been initiated to define the molecular interaction between this purified protein and cellular genetic information during the transformation.

The last 13 years have seen the emergence of a large body of evidence linking the etiology of several human tumors to DNA viruses, especially those of the herpesvirus group. Prior to that time, the only evidence connecting herpesviruses to animal neoplasias was the finding of herpes-like particles in cells of the renal adenocarcinoma of the frog. The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's lymphoma in 1964 stimulated extensive studies to examine herpesviruses as possible etiologic agents of cancer in several animal species, including man. Herpesviruses recovered from wild cottontail rabbits, chickens, and higher primates are now known to be causally related to lymphoproliferative neoplasias in their respective hosts. These and other animal models have provided valuable experimental systems in which to study DNA virus-induced oncogenesis. At least two herpesviruses have been implicated as possible etiologic agents of certain types of human cancer--EBV with lymphoma and nasopharyngeal carcinoma, and Herpes simplex type 2 virus with squamous cell carcinoma of the uterine cervix.

Epstein-Barr Virus. Evidence has been steadily mounting for an etiologic role of EBV in Burkitt's lymphoma (BL) of African children and, to a lesser extent, in nasopharyngeal carcinoma (NPC). The association between EBV and Hodgkin's disease, chronic lymphocytic leukemia, and various non-malignant diseases has been questioned since some patients were found to have no antibodies to EBV and the elevated EBV titers observed in others may reflect the effects of these diseases on the immunological containment of a usually self-limited EBV infection. Finally, it has now been firmly established

that EBV is the causative agent of the heterophile-positive, classical form of infectious mononucleosis (IM), a benign lymphoproliferative disease with predilection for adolescents and young adults.

The Epstein-Barr virus was discovered in the course of an intensive search for viruses associated with BL, a disease which is extremely rare outside of central Africa and parts of New Guinea. The climate-related geographical distribution of this highly endemic disease suggested that insect vectors played some role in its dissemination. The involvement of an infectious agent was strongly supported by the time-space clustering and epidemic drift of the disease. However, the relatively restricted geographical incidence of BL compared to the ubiquitous nature of EBV strongly suggested the interaction of this virus with other co-factors or predisposing conditions to induce this malignancy. A similar pattern is suggested for NPC since this tumor occurs mainly in adults and at an increased frequency in Southern China and certain regions of Africa and Asia where primary EBV infection commonly occurs very early in life.

Seroepidemiologic surveys to study the relationship of EBV to BL and NPC became feasible with the development of tests to detect antibodies to this virus in human sera. Patients were generally found to have elevated levels of antibodies to EB viral capsid antigens (VCA), cell membrane antigens (MA) and to the diffuse (D) or restricted (R) components of the EBV-induced early antigen (EA) complex. The spectra and titers of various EBV-related antibodies have been shown to be of diagnostic or prognostic value. For example, anti-EA activity is primarily directed against the D component in IM and Oriental NPC while antibodies to the R component prevail in BL and Caucasian NPC. Anti-VCA and EA titers are relatively low in the early stages of NPC and in long-term survivors but increase gradually with progression of the disease. Similarly, an unfavorable prognosis for BL patients is indicated by a decline in anti-MA antibody production or an increase in anti-EA titer. Irradiation of BL and NPC *in vivo* has led to an increase of antibody titers against the EB virus-determined MA and VCA. Viral activation followed by antigen release or simple antigen release due to tumor disintegration may explain these effects.

The results of studies on BL and NPC tumor biopsies have been quite consistent. Virus particles, capsid and early antigens have been detected regularly in BL, but not NPC, tumors. An EBV-determined nuclear antigen (EBNA) can be visualized by anticomplementary immunofluorescence in BL and NPC biopsies. The EBNA antigen shows a striking similarity with the T antigens in papova- or adenovirus-transformed cells. Multiple copies of EBV DNA have been demonstrated in virtually every BL and NPC biopsy and tumor cell line examined by molecular hybridization. Furthermore, recent experiments suggest the presence of EBV DNA in epithelial elements of nasopharyngeal carcinomas. This observation is especially important since only cells of the lymphoid series have been shown to undergo transformation by EBV *in vitro*.

EBV has a distinct growth-stimulating effect on lymphoid cells. Exposure of peripheral lymphocytes from normal donors to EBV results in the establishment of continuous lymphoblastoid cell lines. Adult peripheral white cells may

convert into such lines without the addition of extraneous virus, but the derived cell lines usually carry EBV. Several lymphoblastoid cell lines have been established by direct culturing of the BL or NPC itself. In contrast, cultures initiated with lymphoid cells from donors without EBV-directed antibodies or from cord blood or fetal organs failed to grow. The available data indicate that EBV is the major, if not the sole, factor required for establishment of lymphoblast cell lines. Although the antigenic profile and virus productivity patterns vary among these lines, EBV-homologous nucleic acid sequences are, with rare exceptions, always detectable. Expression of the viral genome may be significantly increased by cultivation of the cells in arginine-deficient media, or by exposure of the cells to x-irradiation, mitomycin C, bromodeoxyuridine, or iododeoxyuridine. Thus, the virus genome persists in these cells in vitro, is expressed in varying degrees, and is transmitted during cell division.

Efforts to demonstrate the oncogenic potential of EBV in vivo have met with notable success. Fatal tumors resembling reticulum cell sarcomas were observed in marmosets injected with IM-derived EBV which was propagated in simian cells. In addition, a fatal lymphoproliferative malignancy developed in an owl monkey following injection of cells from an EBV-producing culture of BL origin. If these results can be confirmed and shown to be related to the presence of EBV in the respective inocula, at least two animal models will become available to study the oncogenicity of EBV.

Herpes Simplex Virus. Carcinoma of the uterine cervix is now clearly recognized as the second most common malignant disease of women in the United States. Epidemiologic studies provided the first suggestive evidence that an infectious, venereally transmitted agent was associated with this disease. Later cytohistopathologic, virologic, and seroepidemiologic studies confirmed this observation and identified the suspect agent as Herpes simplex virus type 2 (HSV-2). It is now clear that squamous cell carcinoma of the cervix is related to sexual activity. The pivotal demographic and epidemiologic characteristics that distinguish women at greater risk to developing cervical cancer are low socioeconomic status, early age at first coitus, and number of sex partners.

Seroepidemiologic studies have provided the strongest evidence associating HSV-2 with cervical cancer. Generally, antibodies to HSV-2 have been found more frequently and in higher titer among women with cervical cancer than among control women of similar age, race and socioeconomic level. The antibody activity was found to be age-dependent among control women but not among women with cancer, suggesting that women in the latter group are infected by HSV-2 earlier in life. These differences between patients with cervical carcinoma and controls are more impressive in Black than in Caucasian women and in the U.S., Belgium, and Denmark than in Israel, Columbia, and New Zealand. The occurrence of antibodies to HSV-2 has been noted to increase with progression of the disease; the lowest incidences were found among women with dysplasia and the highest among women with invasive cancer.

Some inconsistencies have been observed in the seroepidemiologic studies and may, in part, be related to shortcomings in the serological specificity of the tests employed. The accuracy with which the present assay methods detect past infections with HSV-2 is unknown. Herpes simplex virus types 1 and 2 appear to share common antigens, and, in addition, have one or more type-specific antigens. Infection with either virus results in the production of antibodies that will crossreact with the heterotypic virus. Since the two types of herpesviruses may possess antigens in common, a prior infection with HSV-1 modifies the production of antibodies to HSV-2. Thus, the criteria used for assessing positivity or negativity for antibodies to HSV-2 may not adequately depict the status of a past infection with this virus. Clearly, the isolation, purification, and characterization of HSV type-specific antigens is crucial to definitive seroepidemiologic studies.

Infectious virus, viral structural antigens, and HSV-specific cytoplasmic changes have not been detected in certical cancer biopsies. However, virion structural antigens were observed in exfoliated tumor cells and tumor cells on the periphery of neoplastic lesions. Further evidence for the persistence of the HSV genome in cervical tumor cells was gathered from in vitro experiments in which infectious virus and viral antigens were detected in spontaneously degenerating cell cultures derived from a carcinoma in situ. Changes similar to those appearing spontaneously could be induced by exposure of the cells to medium of high pH. Virus expression in a culture derived from an invasive cervical carcinoma was limited to a membrane fluorescence of 2% of the cells which could not be augmented by spontaneous or high pH-induced cell degeneration. This data suggests that some cervical cancer cells harbor the complete HSV-2 genome in a repressed state and virus expression occurs following exposure of the cells to condictions of stress.

Photodynamically-inactivated or UV-irradiated HSV-2 has been shown to transform normal hamster embryo fibroblasts in vitro. A variety of serologic tests have yielded evidence for HSV-specific antigens in the cytoplasm and on the surface of the transformed cells. In addition, HSV-specific DNA and RNA have been detected by molecular hybridization in these transformed cell lines. The cells were tumorigenic when inoculated into newborn hamsters and induced virus-neutralizing and membrane reactive antibodies in the sera of tumor-bearing animals. Preimmunization of hamsters with HSV failed to induce transplantation immunity, resulting in enhanced tumor metastases. Transformation in vitro of hamster cells by UV-irradiated HSV-1 and cytomegalovirus and of human embryonic lung cells by heat-inactivated HSV-2 have also been reported.

A key problem encountered in assigning an etiologic role for herpesviruses in human cancer has been their wide distribution in human populations. Tumor formation is usually marked by an increased, rather than a de novo, immunological response to the virus, although antibody titers in cancer patients are much higher than those levels measured in carefully matched normal subjects. Patient immune status to EBV-related antigens correlates with the risk of primary Burkitt's lymphoma or relapse following therapy and with the clinical status of nasopharyngeal carcinoma. The question of why some individuals infected with these viruses develop cancer while others do not remains

unanswered. It appears that EBV expression is controlled at the host level and disseminated following immune impairment. The repressive functions associated with the cell on one hand and the normal host on the other hand might be factors controlling the development of overt lymphomas. It is entirely possible that these viruses act in concert with other biological, environmental, or genetic factors to initiate the neoplastic process. The possible cocarcinogenic activity of human herpesviruses is presently being investigated not only in the United States but in other epidemiologically relevant areas throughout the world.

In the laboratory, experiments are in progress to isolate and characterize herpesvirus-specific nucleic acids and antigens which can be employed as diagnostic or prognostic indicators of tumor development. Attempts are being made to define those regions of the herpesvirus genome which code for cell transformation, to characterize the proteins specified by this genetic information, and to study the interaction of these viral components with normal and malignant cells. The methodology employed in these studies largely reflects that used by tumor virologists studying cell transformation by the papova- and adenoviruses. These include dissection and characterization of the viral DNA with restriction endonucleases and the genetic analysis of mutant viruses which have lost the ability to transform cells in vitro. Progress in this area has been impeded by difficulties encountered in working with such a genetically complex virus. The herpesvirus genome, with a molecular weight of 100×10^6 daltons, is 5 and 25 times larger than the genome of the adeno- and papovaviruses, respectively. Nevertheless, a number of Herpes simplex virus proteins and glycoproteins have been isolated and purified. Among these, VP123 possesses antigenic determinants predominantly specific for HSV-1 and VP119 for HSV-2. New experimental approaches and methodologies are being developed to deal with these problems.

If the herpesviruses are shown to play an etiologic role in human cancer, specific measures can be developed to control their transmission and expression and thereby reduce the incidence of human neoplasia. If these viruses do not act as primary carcinogens, continued study appears warranted based on their role as cocarcinogens and on their utility as specific prognostic and diagnostic aids for certain malignant diseases.

Papovaviruses. Polyoma virus, SV40 and the papilloma viruses are oncogenic members of the papova group of viruses. The capacity of these agents, particularly polyoma virus and SV40, to produce malignant tumors in several different animals is well known. In addition, SV40 has been shown to transform morphologically a variety of cultured cells, including those of human origin. Several papovaviruses of the polyoma-SV40 group have now been isolated from humans. These viruses can be classified into three antigenically distinct groups which crossreact to some extent with SV40 but not with polyoma virus. Since seroepidemiologic evidence has been obtained indicating the widespread colonization of human populations by this group of viruses, their role in human neoplasia is being systematically examined.

b. Progress Highlights

RNA Viruses

Full-length, infectious DNA of Moloney leukemia virus has been synthesized *in vitro*. Infectivity has been found to require at least a segment of plus strand in addition to a full-length minus strand. Inhibition of infectivity by actinomycin D appears to derive from blockage of synthesis of a 5' leader sequence on the glycoprotein in mRNA.

Endogenous RD-114 proviral sequences in cat cells are integrated and covalently bound to chromosomal DNA. However, infectious DNA can be detected only in those cells in which complete virus synthesis is taking place.

The structure of proviral simian sarcoma virus (SSV-1) genes was examined in their native conformation in chromosomes of the NC-37 and A204 human cell lines, which contain 1-3 or 2-6 copies respectively. Ninety percent of the NC-37 but only 50% of the A204 integrated sequences were being actively transcribed. The concentration of viral genes was found to be identical in nucleosomal and total nuclear DNA, clearly demonstrating that the newly integrated virus DNA was organized in a nucleosomal structure in a manner analogous to that of host DNA. Since the genome was actively synthesizing viral RNA, the data further indicated that transcriptionally active regions of the genome remain associated with histone. However, the nucleosomes over the active genes were organized in a different conformation than nucleosomes formed over transcriptionally inert DNA sequences.

The RNA gene product of the murine provirus restriction locus Fv-1 has been partially purified and characterized as 18-22S; perliminary results indicate that the N-tropic viral protein p12 may preferentially bind to this product. In addition, viral gp70 and p30 were shown to be produced in significantly reduced amounts in Fv-1-restrictive infections. Host range conversion from N- or B-tropic to NB-tropic was accompanied by electrophoretic changes in the p30 and Pr65 proteins.

The origin of replication on the genome of Rous sarcoma virus (RSV) has been determined to be approximately 100 nucleotides from the 5' end. Nascent chains are initiated at the 5' end, but elongated toward the 3' end. The terminal redundancy of RSV is 21 bases long, while that of Moloney leukemia virus is 55-65 bases. The mechanism of jumping the gap is still under investigation.

A tridecamer deoxynucleotide complementary to a segment of the 21 nucleotide, 3' and 5' reiterated sequence of RSV 35S RNA has been synthesized, purified, and found to act as a hybridization competitor and inhibitor of virus synthesis *in vitro*.

Full-length cDNA to RSV has been synthesized *in vitro* under conditions allowing high yields of complete, physically intact transcripts of large, polycistronic messenger RNAs. Smaller species of DNA synthesized in these reactions do not represent breakdown products, but rather DNA anti-complementary to the larger

species. This smaller DNA, only 6S in size, contains, in the proper proportions, all the sequences expected from the full-sized complement.

Temperature-sensitive mutants of Rauscher leukemia virus (RLV) are being used to study the relationship between the cleavage of precursor proteins and viral maturation. Two mutants have been found which form late budding structures at the restrictive temperature and accumulate gag and gag-pol gene products. With a shift to the permissive temperature these precursors are cleaved and infectious virus is released.

Most lymphomas, carcinomas, and sarcomas of older domestic cats are negative for the expression of FeLV RNA, p30, or infectious virus. However, regardless of FeLV status, cats with these malignancies show enhanced expression of the endogenous RD-114 genome in neoplastic tissues, although without production of isolatable virus. Normal tissues in these cats show no enhanced expression.

Several histiocytic cell cultures from Hodgkin's disease have been established and characterized. Immunological studies suggest a cross reaction between the p30, gp70 and reverse transcriptase of particles from these cultures and the corresponding proteins from gibbon ape lymphoma virus.

Cells nonproductively transformed by feline sarcoma virus (FeSV) have been found to express the FeLV gag gene-coded proteins, p15 and p12, but not other known FeLV-coded gene products. As with several other previously characterized transforming viruses, the helper virus proteins expressed in these FeSV-transformed cells were translated initially as a precursor whose molecular weight was 125,000 daltons. This precursor was shown to contain FOCMA. Using analogous techniques, cells nonproductively transformed by the replication-defective Abelson lymphosarcoma virus have been shown to express an 85-100,000 dalton polypeptide that contains the MuLV gag gene proteins p15 and p12 covalently linked to an unidentified 60,000 dalton polypeptide. This protein, which lacks antigenic reactivity with other MuLV-coded structural components, is probably the virus-specific transforming protein.

A field vole cell line, transformed by Rous sarcoma virus, has been established. Certain cells in this line revert spontaneously to a normal phenotype. The entire transforming gene sequence is present and biologically active in revertant clones of these cells, indicating that reversion is not due to blockage of transcription. Instead, the vole cells appear to exert post-transcriptional regulation of not only the genes for viral structural proteins but of the transforming gene sequences as well. Investigation of this system is important because it offers a new approach to understanding and potential exploitation of the cell's natural defenses against transformation

Extensive seroepidemiologic investigations have been conducted to assess the risk of human infection by FeLV, particularly since as many as 10% of randomly tested cats demonstrate detectable FeLV antibodies or antigens. A large group of diseased humans, including many with lymphoma or leukemia, were shown to have no immunologic evidence of exposure to FeLV. Similarly, sera from a large number of persons with exposure to leukemic, FeLV-positive cats showed no immunologic evidence of exposure to FeLV, nor did sera from

laboratory workers involved in RNA tumor virus research or from normal controls. It can therefore be concluded, on the basis of these findings, that FeLV infection occurs rarely, if at all, in the human population studied thus far, and that it is unlikely to be a significant cause of disease in man.

Comparative studies on Moloney murine sarcoma virus (MSV) suggest that a large class of defective MSV molecules exists in the uncloned MSV stock. The gag gene polyproteins expressed by each of five clonal isolates of MSV stock were found to be unique in size, ranging from 60,000 to 70,000 daltons. The polyproteins specified by two of the isolates lack p10, indicating that these MSVs have deletions in their 5' p10 gag region. These polyproteins are the largest products synthesized both in cells and in cell-free translation systems. The variable size of the polyproteins expressed by each defective MSV isolate suggests heterogeneity in the size of this gag gene deletion and that it may be similar to the size range that occurs in the dDNA of HSV-1.

Active immunization with inactivated RLV and passive immunization with goat anti-Gross leukemia virus (GLV) IgG reduced the incidence of radiation-induced leukemia in AKR mice and also prevented the expression of endogenous oncornacirus.

The gag polyproteins of two MSV isolates, m1MSV P60 and m3MSV P70, were characterized. The gag gene order was shown to be n-p15-p12-p30-C. Both polyproteins had similar peptide maps from the N-terminus through two-thirds of p30, both lacked p10, and both had different C-terminal peptides. While both m3MSV and Moloney leukemia virus p30 had identical peptide maps, unique peptides were detected in the C-terminal 10 K portion of m1MSV p30, suggesting that the gag gene deletion in m1MSV may begin in the p30 reading frame. In addition to the differences in the size of the gag polyproteins expressed by the two MSV isolates, differences in their biological reversion frequency may indicate a relationship between the gag polyprotein expressed and the stability of the transformed state.

Src sequences have been reproducibly rescued from chemically transformed rat cells and incorporated into a rapidly transforming virus. This represents the first in vitro rescue and incorporation into stable sarcoma viruses of of src genes from tumor cells not infected by exogenous viruses.

Anti-RaLV IgG, added 2-3 days prior to treatment of Fischer rat embryo (F-1706) cells with 4 nitroquinodine (4-NQ), protects the cells from phenotypic transformation and tumor formation in the newborn rat. However, the addition of the antiserum during or after treatment with 4-NQ exerted no protective effect. Preliminary results indicate that anti-RaLV IgG has the same effect when added to cultures of radiation-treated F1706 cells.

DNA Viruses

Numerous herpesviruses have been shown to activate a xenotropic type C virus genome endogenous in mouse cells. Among these are ultraviolet-irradiated pseudorabies virus, infectious bovine rhinotracheitis virus, Herpes simplex virus, and a simian adenovirus, SA8. Furthermore, similar activity was demonstrated by DNA isolated from these agents or Epstein-Barr virus (EBV), human cytomegalovirus, Herpesvirus saimiri, Herpesvirus ateles, and Marek's disease herpesvirus. On the other hand, activating properties were exhibited by DNA extracted from vaccinia virus, SV40, animal cells, bacterial cells, or mycoplasma. It therefore appears that the ability to activate gene expression of endogenous type C viruses in mouse cells is a unique attribute of the herpesviruses. There is, however, presently no evidence that these viruses activate the expression of an endogenous viral genome in human cells.

Epstein-Barr virus-directed nuclear antigen (EBNA) has been purified, giving a single 49,000 molecular weight band on SDS gel. The amount of EBNA per nucleus has been found to be directly proportional to the number of EBV DNA copies in all cell lines and somatic hybrids studied. This indicates that EBNA induction is a relatively autonomous function of the viral genome and is not subject to cellular regulating mechanisms as are other EBV products. Furthermore, persistence or loss of EBV DNA or EBNA from cells could not be associated with any specific human chromosome.

The BK virus (BKV) and JC virus (JCV), papovaviruses for which humans are the natural host, can produce tumors in hamsters. However, in critical studies, no evidence of significant homology has been detected between the nucleotide sequences of BKV and human tumors or continuous lines of cells derived from human tumors. Similarly, indirect immunofluorescence tests showed no evidence of SV40, BKV, or JCV-reactive T-antigen or T-antibody in specimens from cancer patients. These results suggest no significant association between cancer in humans and evidence of infection by these papovaviruses.

As the initial step in elucidation of the enzymes associated with herpesvirus replication, the DNA-dependent DNA polymerases from HSV-1 and Herpesvirus saimiri (HVS) have been purified, and their activities have been compared to the normal cellular DNA polymerases: Both viral polymerases are stimulated by high salt and exhibit normal activity with an oligomer-homopolymer consisting of deoxyguanylate and deoxcytidylate. This preference may be related to the generation of the high G-C dDNA of HVS originating from the ends of the HVS genome.

In studies on SV40 replication in permissive cells, it was found that a substantial fraction of the early RNA is transcribed from free rather than integrated viral templates. However, two separable peaks of activity, revealed by sedimentation anaylsis of early viral transcriptional complexes, suggest the existence of two distinct types of early SV40 templates. It was shown that small amounts of late SV40 RNA can be synthesized prior to DNA replication. This indicates that the transition to predominant synthesis of late RNA occurring after DNA replication is a quantitative phenomenon.

Expression of HSV-2 thymidine kinase (tk) in transformed cells is subject to control by a viral gene not adjacent to the structural gene for tk. "Revertant" clones of all transformed cells appear to have a common DNA sequence of which tk is a part, suggesting a very stable association of the rk region with the cellular DNA.

An AG-4-like antigen from HSV-2 infected cells has been purified and appears to be reactive only with sera from women with cervical cancer. In addition, biochemical and immunologic studies have confirmed the identify of AG-4 antigen with ICP-10. Antibody to AG-4 is found in women with primary HSV-2 infections but infrequently in women with recurrent HSV-2 infections. This is most probably due to the low levels of AG-4 available as immunogen and the 19S nature of the molecule.

A quantitative transformation assay was developed to determine whether specific growth factors, hormones, or combinations of these agents potentiate HSV transformation. Cyclic AMP caused a slight increase in transformation, theophylline caused a marked decrease, and diethylstilbestrol appeared to inhibit transformation.

SV40 mRNAs synthesized during a lytic infection in green monkey kidney cells have been studied and the late 16S and 19S viral mRNA transcripts found in the cell cytoplasm were mapped. A leader of about 210 nucleotides transcribed from one region of the virus genome is spliced to the main coding sequences of 16S mRNA transcribed from a different region of the SV40 genome. Sequences present in the viral genome which lie between these map positions are absent in the cytoplasmic message. Examination of the viral cytoplasmic 19S mRNA identified the genomic map positions of several sequences of nucleotides which are represented as leaders spliced to the same coding sequences of the body of the message. A large fraction of cell nuclear RNA consists of molecules containing the sequences intervening between leader and body which apparently are deleted during processing of transcripts. Nuclear RNA transcripts also are found which extend beyond the terminus of polyadenylated cytoplasmic RNA at 0.17 map units; termination of transcripts at 0.17 map units appears to be associated with polyadenylation.

A newly isolated, highly oncogenic variant strain of Herpesvirus saimiri has been shown consistently to produce malignant lymphoma in owl monkeys and common marmosets after a short incubation period. In other studies, malignant lymphoma, induced by HVS, was observed in cottontop marmosets and owl monkeys which were in direct contact with HVS-infected squirrel monkeys; in addition, purified linear DNA molecules from HVS also induced malignant lymphoma in cottontop marmosets.

Studies of the HMCV herpesvirus, which has biochemical characteristics intermediate to cytomegalovirus (CMV) and HSV, have revealed that in rabbit kidney cells, growth is similar to that of HSV-2; on the other hand, in human diploid fibroblasts, virus growth is like CMV. Both primary human kidney and endometrial cells are transformed by HMCV. The buoyant density of HMCV DNA is slightly higher than the buoyant density of HSV DNA and is far removed from the buoyant density of CMV. The restriction enzyme cleavage

pattern is distinctly different from HSV and CMV. RNA-DNA hybridization studies show less than 10% homology with HSV-1, about 30% with HSV-2, and no homology with laboratory-adapted strains of CMV.

Cellular factors and infection of host cells by type C viruses enhance the growth, but not the transforming ability, of polyoma virus hr-t mutants. Unlike wild type polyoma virus, these mutants do not alter permissive mouse cells so that they can be agglutinated by lectins.

In studies on Marek's disease herpesvirus (MDHV), transformed, non-producer MKT cells were found to contain approximately 25 genome copies of MDHV per cell. These cells, established from a kidney tumor in a chicken with Marek's disease, contained the genome copies primarily as circular plasmid DNA.

Coinfection of chickens with MDHV and high levels of avian leukosis virus results in higher mortality of the birds than does infection with either agent alone. However, tumor induction by MDHV is suppressed by the co-infection.

There is evidence that the cervical epithelium of cebus monkeys infected vaginally with HSV type 2 responds differently from that of control animals. Although the alterations are of a mild nature, the changes are observable and, in some animals, persist.

A previously unknown SV40 gene necessary for malignant transformation has been identified and located at map units 0.54 to 0.59 on the viral genome.

The early events in transformation by SV40 appear to involve an abortive infection of nonpermissive cells. Following infection of mouse embryo cells, which are nonpermissive for SV40, early viral gene products are expressed but late virus-specified antigens do not appear. However, examination of these cells showed that both early and late SV40 genes are efficiently transcribed at a ratio of about 60:40, respectively. This transcription peaks at about 10 hours after infection and drops sharply to low levels thereafter. Since late gene transcripts are detected in the nucleus, the cytoplasm, and in association with polysomes, blockage seems to be at the translational level.

SV40 tsA mutants which make a non-functional T-antigen at the nonpermissive temperature were selected to investigate the function of the early gene products in lytic and transforming infections. At the nonpermissive temperature, the tsA mutants were found to overproduce all the early transcripts and all known early viral proteins in cells lytically-infected as well as in cells transformed by the mutants. One of the early proteins appeared to be preferentially produced in hamster embryo cells transformed by a tsA mutant, suggesting either preferential transcription or processing of the RNA for this protein.

4. Projections

The Viral Oncology Program has set forth a long range research plan to elucidate the role of viruses or viral genes in human cancers and to exploit any leads that develop for use in the detection, diganosis, treatment and prevention of human cancer. The studies are grouped within the following approaches:

a. Virus Studies

(1) Basic Studies. Certain basic studies on RNA and DNA viruses in experimental animals and in cell culture will remain an integral part of the Program. The results of these studies have already provided important information about tumor viruses that is applicable to the isolation and identification of human agents.

(2) Cocarcinogenesis Studies. While it appears that many environmental carcinogens (biological, chemical, or physical) induce cancer, there is evidence that such action is mediated by the activation of latent viruses or viral genes. Cell cultures and suitable methodologies are now available for studying the cocarcinogenic interactions of viruses, chemicals, and physical agents. Such studies will continue to be significantly expanded with the following goals:

(a) to define the role of endogenous viruses in process of cell transformation;

(b) to increase understanding of the relationship of environmental agents as cofactors in carcinogenesis;

(c) to extend and develop new methods of inducing tumor virus expression in "normal" cells;

(d) to develop reliable, sensitive methods for *in vitro* carcinogen testing.

(3) Human Studies. Attempts to isolate RNA or DNA viruses or to detect the presence of viral genetic information and its expression in human tumors will continue. However, considerable emphasis will be placed on developing new probes that can identify viral transforming activity. These efforts will be applied to human cancers:

(a) to identify and isolate viruses, virus expression or viral gene products in human leukemias, lymphomas, sarcomas, and carcinomas;

(b) to apply specific methods for detecting individuals or groups of individuals at high risk to cancer, i.e., individual susceptibility or predisposition to transformation by viruses.

b. Molecular Studies

Increased effort will be given to molecular studies on the mechanisms of interaction of viruses and cells. Major progress has been achieved in understanding the molecular pathways of tumor virus replication. Such advances have provided the basis for sensitive methods for the detection of oncogenic viruses or virus expression. Tests are now available to characterize information detected in normal and tumor cells from patients with various cancers. Since many tumor viruses may be defective, virion replication may be a relatively rare event in human cells. Therefore, emphasis will be given to developing procedures which can detect events of transformation in the absence of viral replication.

(1) Basic Studies. The Program will intensify its efforts to detect, identify and characterize viral genes ("sarc", "leuk" sequences) and viral gene products. Recent studies indicate that whole virus may not be required for converting a normal cell into a cancer cell, and that only one or two viral genes may be involved. Attempts to isolate a "transforming factor", a protein that triggers cells to become malignant, are nearing successful completion.

(2) Applied Studies. As information about the fundamental molecular events in virus-cell interaction becomes available, it will be applied to the study of human cancer:

(a) to develop new probes and sensitive methods for the detection of virus replication and virus transformation;

(b) to identify and characterize viral genes (nucleic acid sequences) and viral gene products (proteins, antigens) found in normal and malignant cells;

(c) to develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral replication and/or tumorigenesis at the cellular and subcellular levels. (The therapy could be directed at any or all of the stages of cell transformation. Ultimately these approaches can be an intensified program to develop anti-enzymes, gene repressors, or other inhibitors effective at the molecular level.)

c. Immunologic Studies

Current trends to concentrate heavily on molecular events in transformation will not detract from the need to pursue immunologic studies. Sensitive, specific methods for the detection of tumor viruses, viral antigens, and tumor antigens found at the cell surface are now available. They are also being applied to understanding the role of immunologic mechanisms in host-tumor and host-virus interactions that may provide a rational approach to the treatment of certain cancers.

(1) Basic Studies. Selected model systems representing tumors induced by RNA and DNA viruses, especially those isolated from non-human primate species, will be studied:

(a) to identify and characterize the viruses, viral antigens and membrane antigens at various stages of the replication and/or transformation process;

(b) to study natural cellular and humoral immune mechanisms in order to determine their relative significance in host recognition of and response to virus-induced tumors;

(c) to develop active and passive immune mechanisms to enhance host response to virus-induced tumors.

(2) Applied Studies. As rapidly as possible, immunological methods will be applied:

(a) to relate putative human viruses or viral information to known oncogenic agents;

(b) to identify and characterize putative human tumor viruses or viral antigens;

(c) to determine the presence of crossreacting antigens suggesting viral causation in various human tumors;

(d) to establish the association of a putative cancer virus with the disease process;

(e) to continue seroepidemiological surveys and case-control studies which will define populations at high risk to cancer, i.e., groups of workers exposed to environmental carcinogens;

(f) to determine the role of host immune responses in virus-induced tumor recognition and rejection;

(g) to develop vaccines (viral or subviral components) from tumor viruses to suppress human cancer.

d. Test Systems and Resources

(1) Test Systems. In vitro and in vivo test systems will be carefully selected to evaluate the work outlined in the previous research areas:

(a) to determine the oncogenic potential of putative human viruses;

(b) to begin viral vaccine (conventional or other) testing and immunization programs;

(c) to explore special animal tumor systems, especially in primate species particularly relevant to human cancer;

(d) to develop and maintain well-characterized cell culture lines and animal stocks (small mammalian and primate species); in particular to develop normal and malignant epithelial cell lines.

(2) Resources. Since research efforts undergo continual change in emphasis and scope as new leads emerge, the Program will continue to develop and maintain a variety of resources:

(a) to collect and store human serum and tissue specimens; integrate data on clinical records, storage and distribution; computerize specimen collection;

(b) to maintain various mammalian animal colonies, especially endangered primate species, for basic research and special studies;

(c) to develop methods to produce candidate human viruses as they are isolated;

(d) to produce large amounts of animal tumor viruses and high quality diagnostic reagents for research;

(e) to provide containment facilities for research on known oncogenic viruses and candidate human tumor viruses; in particular, to provide facilities for conduct of recombinant-DNA studies as appropriate.

5. Reports on Information Exchange Activities, International Agreements, and Meetings Sponsored by the Viral Oncology Program

(Fiscal Year 1978)

International Agreements

US-USSR Agreement on Health Cooperation - Joint Working Group on Cancer Virology

US-France Cooperation in Cancer Research - Committee on Cancer Virology (with emphasis on immunology)

US-Japan Cooperative Cancer Research Program

Meetings Sponsored or Partially Sponsored by the Viral Oncology Program (VOP)

Twelfth Annual Joint Working Conference - VCP (Hershey, PA; November, 1977)

Workshop on the Utilization of EBV Reagents in the Diagnosis and Prognosis of NPC (Bethesda, MD; December, 1977)

International Symposium on Papovaviruses and Their Role in Cell Transformation and Oncogenesis (Bethesda, MD; February, 1978)

Fourth Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy (Bethesda, MD; March, 1978)

11th Meeting on Mammary Cancer in Experimental Animals and Man (Detroit, MI; June, 1978)

Fourth International Congress for Virology (The Hague; September, 1978)

Meetings to be Sponsored by the Viral Oncology Program

Thirteenth Annual Joint Working Conference - VOP (Hershey, PA; November, 1978)

IXth International Symposium on Comparative Research on Leukemia and Related Diseases (Sukhumi, USSR; October, 1979)

US-USSR Agreement. A Memorandum of Understanding for cooperation in the study of the microbiology, immunology, and molecular biology of cancer viruses was first signed on November 18, 1972. The Memorandum established procedures for joint studies through the exchange of information, materials and scientists between the two countries.

Delegation Meetings:	November, 1972	Moscow, USSR
	November, 1973	Bethesda, USA (Subcommittee)
	May, 1974	Moscow, USSR
	May, 1975	Bethesda, USA
	June, 1976	Sukhumi, USSR
	October, 1977	Bethesda, USA
	September, 1978	Riga, Latvian SSR

As agreed, the fifth meeting of the US-USSR Joint Working Group on Cancer Virology, Co-Chairmen Dr. J.B. Moloney and Professor V.M. Zhdanov, took place at the National Institutes of Health, Bethesda, Maryland, USA, on October 26-28, 1977. At a symposium held on October 27 and 28, members of both delegations and invited speakers presented recent studies in cancer virology. The main emphasis of this meeting was given to reviewing the progress of current cooperative efforts and assessing the problem of recombinant DNA research. Dr. Michael Crawford (University of Kansas) presented preliminary results of a study to determine the role of genetic factors in an outbreak of leukemia in baboons. This work, conducted jointly by laboratories in the USA and in the USSR, is an excellent example of the cooperative research efforts sponsored under the US-USSR Agreement.

The Chairmen of both Sides reported on the recommendations made in the Memorandum of Understanding of the Joint Committee on Malignant Neoplasia held in Moscow, USSR, September, 1977. The recommendations included: (1) discussing, in depth, cooperative studies on recombinant DNA research, (2) increasing the program participation of other USSR institutions, in particular to include the Institute of Molecular Biology, Moscow, (3) conducting exchanges of scientists only under the auspices of the Cancer Virology Program under the topic of Malignant Neoplasia, USA-USSR Health Agreement, and (4) encouraging the use of small working group meetings on subjects of intense interest.

Delegates expressed interest in conducting collaborative studies in the following areas: (1) studies of viruses isolated from human tissues in cell culture or in animals and their possible role in the pathogenesis of human neoplasia; (2) continuation of studies on non-human primate viruses as they relate to human cancer; (3) studies on the role of viruses in the induction of human breast tumors, including continuation of studies on MPMV and related viruses; (4) studies on cocarcinogenesis--viral/viral, viral/chemical, and viral/hormonal; (5) characterization of nucleic acids and their role in the induction of animal and human cancers, particularly the detection of transforming sequences in cellular nucleic acids and molecular genetic studies with DNA from human tumor cells; (6) studies on viral proteins as probes for viral gene expression in animals and humans; and (7) studies on oncogenic viruses important to human ecology, e.g., those derived from bovine, avian,

and feline species. These areas essentially represent a continuation of cooperative research efforts stated in previous Memoranda. In addition, both Sides accepted the possibility that application of techniques for recombinant DNA research, methods for structural and functional studies of genomes, of oncogenic viruses, studies of the mechanism of neoplastic processes, and the search for fundamentally new approaches to prophylaxis and therapy of cancer might be applied to problems of cancer virology, subject to the guidelines developed by the U.S. National Institutes of Health in 1976. These guidelines were adopted by the scientific community pending the enactment of legislation governing such experimental work.

Both Sides reaffirmed the necessity to certify the exchanges of scientists and research reagents through the Offices of the respective Chairmen. Both Sides also recognized the importance of continuing the exchange of scientists for conducting joint research and again stressed the need for exchange of young scientists.

Future Meetings: American and Soviet tumor virologists will continue to participate in relevant conferences in their respective countries. It was proposed that the sixth meeting be held in Riga, Latvian SSR, in September, 1978.

Summary of US-USSR scientist exchange program to date:

TO USSR:		INSTITUTIONS VISITED
Dr. Walter Nelson-Rees University of California, Berkeley	November, 1974	Inst. Expt'l Clinical Oncology Inst. Medical Genetics Inst. Viral Preparations Inst. Virology Inst. Epidemiology (Gamaleya)
Dr. Robert Goldberg Viral Oncology Program, NCI	May, 1976	Inst. Expt'l Pathology and Therapy, Sukhumi (Dr. Lapin) Institutes in Moscow and Leningrad
Dr. John Cicmanec Litton Bionetics, Inc. Kensington, Maryland	May, 1976	Inst. Expt'l Pathology and Therapy, Sukhumi (Dr. Lapin) Institutes in Moscow and Leningrad
Dr. D.H. O'Rourke Dr. R.M. Baume University of Kansas, Lawrence	June, 1977	Institute of Experimental Pathology and Therapy, Sukhumi (Dr. Lapin)
Dr. Sami Mayyasi Pfizer, Inc. Maywood, New Jersey	September, 1977	Inst. Expt'l Pathology and Therapy, Sukhumi (Dr. Lapin) Institutes in Moscow and Leningrad

TO USSR (continued):		INSTITUTIONS VISITED
Dr. Charles Boone Viral Oncology Program, NCI	September, 1977	Institutes in Moscow, Kiev, Riga, Leningrad
Dr. S. Hakomori University of Washington, Seattle	June, 1978	Inst. for Oncology Problems, Kiev; Shemyakin Inst. of Bioorganic Chemistry, Moscow
Dr. Michael Crawford Dr. Dennis O'Rourke University of Kansas, Lawrence	August, 1978	Institute for Experimental Pathology and Therapy, Sukhumi (Dr. Lapin)
Dr. Bernard Roizman University of Chicago	Summer, 1978	Institutes in Moscow and Leningrad
Dr. Harvey Rabin Litton Bionetics, Inc. Kensington, Maryland	August, 1978	Institute for Experimental Pathology and Therapy, Sukhumi (Dr. Lapin)
TO U.S.		
Dr. Viktor Zhdanov Ivanovsky Inst., Moscow	October, 1973	NCI scientists: VCP Annual Meeting
Dr. I.F. Seitz Petrov Research Institute, Leningrad	October, 1973	NCI scientists: VCP Annual Meeting
Dr. Konstantin Ilyin Gamaleya Institute, Moscow	February, 1974	NCI scientists: Flow Labs., Rockville; M.D. Anderson Hospital, Houston
Dr. T.A. Bektemirov Ivanovsky Inst., Moscow	October, 1974	NCI scientists: A. Einstein Coll. Med. (Dr. August); Columbia Univ. (Spiegelman)
Drs. E. Dzhikidze Z. Shevtsova V. Agrba Yakovleva Inst. Expt'l Pathology, Sukhumi	September, 1975	NCI scientists: VCP Annual Meeting; laboratories of Drs. Deinhardt, Spiegelman, Mayyasi; Sloan-Kettering Institute
Dr. N. Nosik Ivanovsky Inst., Moscow	September, 1975	Litton Bionetics (Dr. Fine); VCP Annual Meeting; Baylor Univ. (Dr. Melnick); Pfizer, Inc. (Dr. Mayyasi)

TO U.S. (continued)		INSTITUTIONS VISITED
Dr. I. Kryukova Gamaleya Inst., Moscow	February, 1976	M.D. Anderson Hosp. (Dr. Bowen); Michigan Cancer Fdn (Dr. Rich); NCI scientists; Rockefeller Inst. (Dr. Hanafusa)
Prof. S.M. Klimenko Ivanovsky Inst., Moscow	September, 1976	NCI scientists; Inst. Cancer Research (Dr. Blumberg)
Dr. E. Bagley Kiev Inst. Experimental and Clinical Oncology	March, 1977	NCI scientists; F. Hutchinson Cancer Ctr (Dr. Hakomori); Sloan-Kettering Institute (Dr. Sonnenberg)
Dr. Z. Butenko Kiev Inst. Experimental and Clinical Oncology	March, 1977	NCI scientists; laboratories of Drs. Spiegelman, Mayyasi, W. Moloney, E. Cronkite
Dr. S.A. Novakhatskiy Ivanovsky Inst., Moscow	May, 1977	NCI scientists; laboratory of Dr. R. Gallo, NCI; area laboratories involved in large-scale production of human virus
Dr. Felix Filatov Ivanovsky Inst., Moscow	September, 1977	University of Chicago (Dr. B. Roizman)
Dr. L.B. Stepanova Dr. O.B. Korchak Moscow Research Institute of Viral Preparations	November, 1977	NCI Laboratory of Viral Carcinogenesis, Viral Oncology Program
Prof. I.F. Seitz Petrov Institute of Oncology, Leningrad	April, 1978	NCI (Dr. Gallo); USC (Drs. McAllister and Vogt); UCLA (Baluda); Sloan-Kettering (Dr. Bendich)
Dr. Boris Lapin Director, Inst. for Experimental Pathology and Therapy, Sukhumi	September, 1978	NCI scientists; Sloan-Kettering Inst. (Dr. Moore-Jankowsky); Delta Regional Primate Ctr (Dr. Gerone)

Dr. Felix P. Filatov, Senior Scientific Researcher, Ivanovsky Institute of Virology, Moscow, spent three-and-one-half months in the laboratory of Dr. Bernard Roizman, University of Chicago. The purpose of his exchange visit was to gain experience in (a) preparative purification of Herpes

simplex virus DNA, (b) analysis of the DNA by restriction endonucleases, (c) preparative-scale purification of DNA fragments generated with restriction endonucleases and (d) analyses of biologic activity of restriction endonuclease fragments.

Dr. Lidia G. Stepanova and Dr. O.B. Korchak, members of the staff of the Moscow Scientific Research Institute of Viral Preparations, spent the months of November and December, 1977, in various NCI laboratories, primarily working in a laboratory in the Virus Containment Facility of Building 41 under the guidance of Dr. Arthur Frankel, Laboratory of Viral Carcinogenesis. The goals of the visit were to (a) become familiar with the American technology for viral characterization through nucleic acid hybridization and radioimmunoassay, (b) characterize and compare the properties of the "LPV oncornavirus" of USSR origin with certain primate viruses, and (c) observe and compare the technology for the mass production of cells and viruses. Before leaving the U.S., Drs. Stepanova and Korchak spent three days in the laboratory of Dr. Sol Spiegelman, Columbia University Institute for Cancer Research in New York City.

Dr. Sami A. Mayyasi, Assistant Director of Cancer Research, Pfizer, Inc., Maywood, New Jersey, visited the USSR in September, 1977 for two weeks to discuss methodology for the large-scale production of oncogenic viruses, to study type C viruses, and to review Mason-Pfizer monkey virus morphology and immunology in relation to other simian viruses. Institutes included in his itinerary were the Cancer Research Center (Moscow), the D.I. Ivanovsky Institute of Virology (Moscow), the Institute of Experimental Pathology and Therapy (Sukhumi), and the N.N. Petrov Institute of Oncology (Leningrad).

Dr. Charles Boone, Head, Cell Biology Section, Laboratory of Viral Carcinogenesis, spent three weeks in the Soviet Union in September-October, 1977. The purpose of his visit was to present work on "virus-augmented" human melanoma skin test antigens at various cancer institutes in the USSR, and to evaluate in depth the work of A.J. Munceniece on the virus therapy of cancer. Institutes visited were: Cancer Research Center (Moscow); Hertsen Institute of Oncology (Moscow); the N.N. Petrov Institute of Oncology (Leningrad); the August Kirchenstein Institute of Microbiology in Riga (3-day visit with Dr. A.J. Munceniece and staff); and two institutes in Kiev--the Institute for Problems in Oncology (Dr. Julian Umansky) and the Institute for Roentgenology and Radiology (Dr. Anatoley Bukorez).

US-France Cooperation. Exchange of information and of scientists between the United States and France has been greatly expanded with the initiation of a formal cooperation between INSERM and the NCI in December, 1975. Three major areas are involved: (1) Viral Oncology, (2) Hormone Control and Cancer, and (3) Clinical Trials for Cancer Treatment. The respective Program Coordinators for Viral Oncology, Dr. J.P. Levy (France) and Dr. J.B. Moloney (U.S.), co-chaired the second joint meeting of the Viral Oncology Delegations held in Paris on September 28-30, 1977. There was general agreement that much had been accomplished during the first year. On the first day, the French Side submitted a compendium of French scientists

working in the area of viral oncology. This document should prove to be helpful in promoting the scientific cooperation. A second report on the state of recombinant DNA studies indicated that the posture of the French on this subject will probably be similar to that of the U.S. A symposium was held on the second and third days of the meeting.

Some discussion centered on the duration of exchange assignments. It was agreed to maintain flexibility of duration and numbers of visits, the only limits being commitments of total man-months. It was recommended that exchange scientists prepare summary reports to be submitted to their respective chairmen, and that future joint meetings should include presentation by these scientists of their work. Both delegations agreed to continue collaborative research in the areas of RNA tumor viruses, DNA tumor viruses, and the role of viruses in human cancers.

The next joint meeting was set for Fall, 1978, in the United States.

Summary of US-France scientist exchange program to date:

TO FRANCE

Dr. M. Hatanaka (NCI) to Inst. Pasteur (P. Hosli) - April, 1977 (3 months)

Dr. N. Salzman (NIAID) to IRSC, Villejuif (R. Monier) - June, 1977 (6 weeks)

Dr. C. Sherr (NCI) to Hopital St. Louis, Paris (J. Peries, A. Tavitian) - October, 1977 (3 weeks)

Dr. R. Glaser (Penn State Univ.) to Inst. Pasteur (S. Michaelson-Fiske) - November, 1977 (3 weeks)

Dr. L. Warren (Wistar Inst.) to Inst. Pasteur (L. Montagnier) - August, 1977 (10 months)

Dr. S. Oroszlan (FCRC) to Paris (Vignier), Lyon (Huppert), Paris (Levy) - January, 1978 (3 weeks)

TO U.S.

Dr. L. Gazzolo (INSERM, Lyon) to V.A. Hospital, Gainesville, Florida, (C. Moscovici) - July, 1977 (2 months)

Dr. J. Seigneurin (Grenoble) to NINCDS (Dubois-Dalcq) - July, 1977 (3 months)

Dr. M. Yaniv (Inst. Pasteur) to Stanford Univ. (P. Berg) - July, 1977 (3 months)

Dr. J. Youn (Inst. Gustav-Roussy) to Microbiological Associates, Bethesda, MD. (J. Rhim) - Sept.,1977 (2 months)

TO U.S. (continued)

Dr. R. Vigne (Secteur Univ., Marseille) to USC (P. Vogt) - Oct., 1977 (3 months)

Dr. E. Gomard (Hopital Cochin, Paris) to NCI (G. Shearer) - Nov., 1977 (2 months)

Dr. J. Gogusev (College de France) to NCI (U. Heine) - Nov., 1977 (5 months)

Dr. M. Favre (Inst. Gustav-Roussy) to St. Louis Univ. (M. Green) - Feb., 1978 (2 months)

Dr. B. Varet (Hopital Cochin) to State Univ. N.Y., Buffalo (G. Cudkowicz) - April, 1978 (3 months)

Dr. Evelyne May (CNRS) to NCI (G. Khoury) - June, 1978 (7 weeks)

Dr. G. Calothy (Fondation Curie) to Rockefeller Univ. (H. Hanafusa) - July, 1978 (1 year)

Dr. F. Cuzin (Univ. of Nice) to Harvard Univ. (T. Benjamin) - June, 1978 (2 mo.)

During this fiscal year, Dr. Upton appointed Dr. Sibal to assume the responsibilities of Chairman of both the US-USSR Cancer Virology Delegation and the Working Group of the US-France Health Agreement. The members of these groups are:

US-USSR Cancer Virology Delegation:

Dr. Louis R. Sibal (Chairman), National Cancer Institute, Bethesda, MD
Dr. Stuart Aaronson, National Cancer Institute, Bethesda, MD
Dr. J. Thomas August, Johns Hopkins University, Baltimore, MD
Dr. Dani Bolognesi, Duke University, Durham, NC
Dr. Robert M. McAllister, University of Southern California, Los Angeles, CA
Dr. Robert A. Manaker, National Cancer Institute, Bethesda, MD
Dr. Fred Rapp, M.S. Hershey Medical Center, Hershey, PA
Dr. David Yohn, Ohio State University, Columbus, OH

Working Group, US-France Health Agreement

Dr. Louis R. Sibal (Chairman), National Cancer Institute, Bethesda, MD
Dr. Janet S. Butel, Baylor College of Medicine, Houston, TX
Dr. Raymond Gilden, Frederick Cancer Research Center, Frederick, MD
Dr. Fred Rapp, M.S. Hershey Medical Center, Hershey, PA
Dr. Marvin A. Rich, Michigan Cancer Foundation, Detroit, MI
Dr. Edward Scolnick, National Cancer Institute, Bethesda, MD

US-Japan Cooperation. This cooperation was established in 1974 under an Agreement between the National Cancer Institute and the Japan Society for the Promotion of Science (JSPS). The Program Coordinators for Cancer Virology are Professors Robert McAllister (USA) and Yohei Ito (Japan). A Symposium on the Role of DNA Tumor Viruses in Cancer was held in Honolulu, Hawaii on May 22 and 23, 1978. The major purpose was to exchange information and encourage cooperative experiments among Japanese and American virologists working particularly on the mechanisms of oncogenesis by DNA tumor viruses at the molecular and cellular levels and in the host. Attempts were also made to correlate the recent basic findings in the research area with the efforts to determine the etiological role of DNA viruses in certain types of animal and human neoplasia. Participants included nine American and seven Japanese scientists.

MEETINGS

Twelfth Annual Joint Working Conference - Virus Cancer Program. The annual meeting of the Virus Cancer Program was held at Hershey, Pennsylvania on November 1-4, 1977. Topics discussed were: new primate retroviruses; precursors, polyproteins, and membrane antigens; helper-independent and defective viruses of murine origin; herpesviruses and cancer; molecular studies of viral cell interactions; new developments and approaches in oncornavirus studies; new approaches in DNA tumor virus studies; new approaches and new directions in cocarcinogenesis; new approaches and new directions in clinical studies; new approaches and new directions in breast cancer virus studies; and mechanisms involved in virus-induced leukemia. The official program of this meeting may be obtained from this Office upon request.

Workshop: The Utilization of EBV Reagents in the Diagnosis and Prognosis of Human NPC. This meeting, held at the NCI on December 12-13, 1977, and sponsored by the VOP, was chaired and organized by Dr. Robert Goldberg, Laboratory of Tumor Virus Genetics, VOP, DCCP, NCI. The Workshop was convened to answer the following questions: (1) Can EBV-specific reagents be used for the diagnosis and prognosis of human NPC? (2) Should an NCI-sponsored clinical study be initiated in the U.S. at this time to test this question? (3) What is the best protocol for an effective and comprehensive clinical study?

The meeting included presentations by invited participants in which the histopathology, immunology, virology, epidemiology, radiotherapy, staging, and management of NPC were reviewed. Extensive discussions followed each talk. On the second day, Dr. Dharam Ablashi presented an in-depth review of the recommendations generated from the International Symposium on Etiology and Control of NPC held in Japan in April, 1977. The workshop ended with a planning session in which the optimal parameters for an NCI-sponsored clinical study were defined.

The participants were unanimous in recommending an NCI-sponsored, comprehensive multidisciplinary program in the U.S. to validate the utility of EBV immunologic

studies in the diagnosis and prognosis of human NPC. All participants urged the rapid initiation of such a study since at least five years would be needed to place this approach in a clinically useful context. Specific recommendations offered were: 1) Clinical participation should be predicated upon such factors as patient number and accessibility, geographical distrubution, and commitment to logistical coordination; 2) testing must be a multidisciplinary approach involving immunological, histopathological and virological evaluations; 3) a uniform system of classification for tumor staging should be used and clinicians should submit enough relevant diagnostic data to allow patient assignment within this system; 4) a comprehensive form should be designed for recording all essential clinical and laboratory data on each patient and the data should be integrated into a computer-based storage system suitable for rapid retrieval, collation, and analysis; 5) primary serological, virological and histopathological analyses should be performed in a "magnet" or "primary" laboratory with expertise in these areas. Serological confirmation of anti-EBV titers should be obtained from an independent "secondary" laboratory.

International Symposium on Papovaviruses and Their Role in Cell Transformation and Oncogenesis (February 1-3, 1978). This symposium jointly sponsored by the Viral Oncology Program and NIAID, was held at the NIH and included sessions on (1) structure of the viral genome sequences (genetics and biologic function), (2) viral polypeptides, (3) transformation, (4) integration and excision, (5) transcription, and (6) DNA replication. Approximately 50 scientists working in the DNA virus field participated in the symposium.

Fourth Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy. Held on March 29-31, 1978, at the NCI, this meeting included a series of invited papers on the use of immunoadjuvants in cancer therapy. Sessions were devoted to each of the following adjuvants: levamisole, thymosin, maleic anhydride-vinyl ether copolymers, glucan, interferon, and brucella abortis extract. Papers were also delivered on the effects of thiobendazole, isoprimosine, krestin , and BM 12.531 in immunostimulation. Particular attention was given to levamisole; this compound has been increasingly used in research on combined modality therapy of various forms of human cancer.

Meeting on Mammary Cancer in Experimental Animals and Man (June, 1978 - Detroit, Michigan); Fourth International Congress for Virology (September, 1978 - The Hague, The Netherlands). Brief accounts of these meetings will be given in next year's annual report.

IXth International Symposium on Comparative Research on Leukemia and Related Diseases (October, 1979). This meeting, to be held in Sukhumi, USSR, will be described in next year's annual report.

B. 1. a. OFFICE OF BIOHAZARD SAFETY

A major problem encountered by all areas of biomedical science is the lack of suitable facilities for the performance of research with infectious agents such as viruses and toxic chemicals such as carcinogens. Facilities must offer the environmental controls necessary for protecting laboratory personnel, experimental animals and the surrounding community from exposure to potentially hazardous material. Associated with suitable facilities are properly trained personnel required to operate in such facilities to maximize their potential capabilities. The Office of Biohazard Safety (OBS) provides the administrative and technical expertise for the development of facilities and techniques to minimize potential exposure to toxic substances. In addition to defining the capacity of contractor and grant supported laboratories to meet and implement federal safety standards for working with infectious agents and chemical carcinogens and mutagens. Therefore there exists a requirement for field evaluation, performed by personnel of the office who are active research scientists, having both theoretical and practical knowledge in laboratory safety and current research practices.

Risk assessment associated with the use of infectious agents and chemical mutagens and carcinogens is evaluated on a continual basis as new data becomes available. Parameters employed for such an assessment is the route of transmission, host range, antigenicity of an infectious agent, as well as the capacity to replicate and integrate into the potential host genome. Also considered are such points as agent concentration, techniques employed in the laboratory working with the agent as well as the physiological state of the potential host. Since the use of animals, in particular nonhuman primates, in the biomedical laboratory has become prevalent, a number of heretofore unanticipated problems have arisen. The inadequate care and handling of animals during the past several years have created a potential for the occurrence of infection of humans with simian microorganisms, infection of simiae with human microorganisms and cross-infection between simian species. Such interspecies disease transmission may seriously compromise the integrity of the experiment as well as the health of the experimentor. To assess problems associated with the use of microbial and chemical agents as well as the employment of experimental animals, information on how best to reduce potential risk is obtained by personal experience, literature search, and the convening of expert consultants to assess risk potential and arrive at logical and workable means for reducing such potential risk.

This office, after evaluation, disseminates this information to researchers potentially exposed to such risks, so that workable control measures may be instituted. Educational and technical assistance on cancer research safety matters as well as laboratory data developed to demonstrate the efficiency of such practices are collated and distributed to the scientific community. It is recognized that the task of prescribing protective measures to prevent laboratory acquired pathological consequences is often arbitrary and difficult

to substantiate by hard data, particularly since most of the carcinogenic agents under investigation have not been definitively implicated in the etiology of cancer in man. In the case of the tumor viruses it is still in the realm of possibility that during the course of laboratory experimentation to further comprehend cell biology, an agent infectious to man may be isolated. In view of this possibility, it is necessary that adequate protective measures be continually applied and refined to assure the lay community as well as the scientific community that they are not inadvertently being exposed to oncogenic and mutagenic agents. This is best accomplished by practicing proper laboratory procedures which as a bonus also reduce the possibility of cross-contamination of laboratory experiments. Proper techniques are augmented by safety equipment such as pipetting aids, face masks, gloves, and biological safety cabinets. Data for the design of such equipment are obtained by the performance of applied experimentation in the areas of air mass movement, air dispersion and dilution as well as particulate and gaseous matter retention.

Due to the magnitude of biomedical research employing tissue cultures, frequent evaluation of tissue culture cross-contamination is very important. This office obtains data on the mechanisms that permit such cross-contamination and how best to prevent such occurrences. Techniques are currently under development to address specialized biohazard problem areas in viral oncology and chemical carcinogenesis. Animal holding facilities are being examined to identify those practices which permit normal animal and human microbial flora from being inadvertently transmitted. The office serves as a focus for the collection, maintenance and testing of sera obtained from laboratories working with infectious oncogenic agents. Information derived from such studies are analyzed and integrated into a medical monitoring computer system. Pertinent data obtained from such endeavors are disseminated to interested parties. Medical evaluation of in-house staff by means of medical examination, laboratory evaluation and information integration is an ongoing process.

To further minimize laboratory associated cross-contamination, periodic surveillance of laboratories is carried out. Special emphasis is directed at resources production facilities in order to minimize cross-contamination that could lead to mass confusion. Renovation and construction plans are reviewed by the office to assure the facilities funded by federal monies meet safety standards. All contract proposals are reviewed by this office to assure that protocols and facilities minimize potential hazards. In order to obtain laboratory data associated with safety practices as well as to maintain a staff that is current in broad areas of biomedical research, this office has assigned to it a research section.

The Biohazard Research Section (BRS) is responsible for developing technical information that permits the OBS to make certain recommendations based on scientific data and to maintain a staff aware of the most recent technical procedures and scientific data. This is also the only laboratory in the in-house viral oncology program developing a data base utilizing both animal and in vitro model systems to evaluate the host's physiological contribution to the viral-hose interaction. Current studies stress endogenous type C virus

interaction with immunological defense mechanisms, reproductive process and chemical carcinogens. Mouse, endogenous, xenotropic as well as ecotropic, type C virus replication is activated by chemical and immunological means. The presence of the type C viruses seems to be associated with a wide variety of immunological phenomena including transplantation and graft versus host reactions and autoimmune disease in vivo and mixed lymphocyte reactions in vitro. Type C virus induced immunodepression is well documented and frequently studied as a model of immunosurveillance breakdown. Virus activation, replication and pathogenicity is genetically controlled within or is closely linked to the major histocompatibility complex and viral antigens have been shown to be physically associated with H-2 antigens on tumor cell surfaces. Furthermore, cellular immune destruction of virus-infected cells in the mouse requires compatibility between target and effector cells at loci within the H-2 complex. Also, viral envelope glycoproteins (gp70) are expressed on the cell surface of normal as well as malignant thymocytes in many strains of mice. This suggests that, in addition to their oncogenic potential in many species, type C viruses may be intimately involved in immunological recognition processes at the level of the plasma membrane. This may be particularly true of the apparently nonpathogenic xenotropes' gp70 studied in this laboratory and is supported by the recently reported similarities between surface gp70. This includes the examination of murine and primate xenotropic virus, looking for evidence of their pathogenicity in their host of origin and heterologous species, including man, and describing examples of their participation in immunoregulatory processes. To achieve these goals, humans and baboons are being examined for immunological evidence of endogenous or exogenous infection with the baboon virus M7 or cross reacting viruses. Evidence of humoral or cellular immunity as well as for the presence of viral associated or virus coded antigens is being obtained by immunological assays. Techniques are being developed and used to study the mechanisms of type C virus or viral protein modification of immune recognition processes at the effector and/or target cell level in murine and primate species. The most significant advance during this year has been the isolation and purification of a type C viral associated protein, which modifies the in vitro and in vivo cellular immune mechanism. We are suggesting that differential activation of such endogenous viral associated proteins could serve an immunoregulatory function under normal physiological conditions. The binding to specific lymphoid cell receptor sites has been examined in great detail and is currently being extended to determine whether cell surface modulation, induced by these proteins, will modify cellular activation and responses to nonspecific mitogens and antigens. Toward this end we have established several in vitro systems to evaluate the influence of leukemia cells, type C virion or subvirion proteins on cell recognition processes. The major envelope glycoprotein of R-MuLV binds rapidly and with great specificity to mouse lymphoid cells. Murine ecotropic virus but not xenotropic viruses interfere with R-MuLV adsorption, indicating that murine cells contain a population of receptors for ecotropic virus. The relative binding capacity of cells from different strains of mouse spleen, the primary target of R-MuLV exhibit a much greater binding capacity dependent on

strain than do mouse sperm or erythrocytes, suggesting differences in binding capacity for different organ and mouse strains. The active binding region for the gp70 molecule is associated with a protein of less than 30,000 daltons. Whereas the binding kinetics are similar to the native glycoprotein, the divalent cation requirements for optimum binding are less stringent. Currently, three major cell surface proteins have been isolated and appear to be associated on the cell surface and influence virus interaction. The modulation of cell surface receptors and antigens in mouse T and B lymphocytes is being investigated by studying their distribution on cell membranes and their redistribution induced by a variety of ligants in capping experiments. We have found that Friend virus leukemia cells inhibit capping of normal mouse cells by H-2 alloantisera and anti-mouse IgG. The influence of subvirion proteins on capping is questionable, but antisera to some of these proteins (e.g. anti-gp70) do inhibit. Binding of viral proteins and their subsequent influence on lymphoid cell function requires an initial interaction between the protein and specific cell receptors. A thymocyte, plasma membrane protein that prevents binding of subvirion proteins to mouse lymphoid cells has been isolated and is currently being characterized by biochemical, immunological and biological means. Other areas being investigated include the presences and function of low molecular weight host cell DNA, possibly adjacent to provirus integration sites. Also peptide mapping of p30 for different type C viruses are being looked at to determine if this very sensitive technique may provide means for the grouping of type C viruses into classes and the evaluation of some of the more physiologically active proteins currently under study in our laboratory and involved in immunodepression and cell membrane modification. Information obtained from these types of studies will permit a more definitive evaluation of the pathological as well as physiological function of type C viruses and their significance to mammalian biology.

SUMMARY REPORT

1. b. FREDERICK CANCER RESEARCH CENTER

Introduction: The Viral Oncology (VO) Program at the Frederick Cancer Research Center (FCRC) will have essentially concluded six and one-quarter years of operation on September 25, 1978. The overall objective of the FCRC/VO Program is to conduct a comprehensive investigation on oncogenic and suspected oncogenic viruses and their interactions with host cells to determine the potential carcinogenic or co-carcinogenic role these agents may play in the neoplastic process, and to develop therapeutic and preventive measures for their control.

Following an interval of program assessment and the subsequent implementation of several preliminary steps, two years ago last June NCI/VO introduced a plan that provided for an extensive reorganization of the VO effort at its Frederick facilities. In essence, this plan called for doing away with the Task or Project structure in favor of one that provided for a more flexible and comprehensive response to NCI/VO Program needs and requirements. Thus, the program essentially was to consist of a substantial new research effort along with an increased resource activity to accommodate the increased emphasis on the former. The new structure consisted of RNA Virus, DNA Virus, and Disease Control Laboratories and a Viral Resources Laboratory. Of critical importance from the standpoint of the reorganized program development, was the addition to the Contractor's staff of the Director of Viral Oncology (DVO). This individual, well recognized in the scientific community for his scientific and administrative capabilities, joined the VO/FCRC program in January of 1976. Additionally, throughout the first half of that year, the new DVO successfully attracted a number of capable scientific personnel for strengthening new and ongoing effort. The arrival of the DVO marked the positioning of an important cornerstone around which NCI's plan for significant development of the VO/FCRC program could be brought to fruition, and since that time, many important achievements have been made.

Attending the above program changes, a fifth Laboratory effort was initiated in 1976, namely, the Director of VO Program, which involved work in immunochemistry and in oncornavirus immunobiology, providing important adjuncts to research in the other Laboratories. Of great importance also was the initiation of a new era of cooperation between Contractor and on-site NCI senior staff investigators, resulting in each performing a mutually beneficial role within the FCRC/VO sphere of activities. Moreover, valuable collaborative efforts were initiated with a number of recognized outside scientists whose expertise advantageously augmented the VO effort. Also, with members of the NCI and Contractor's staff, facilities were planned and renovated and/or improved to provide highly suitable laboratories for conducting the VO program.

During 1977, the area referred to above as the Director of VO Program, was redesignated as the Immunochemistry Laboratory, consisting of work in immunochemistry and oncornavirus immunobiology. This Laboratory then took its place along with the RNA Virus, DNA Virus, Disease Control and Viral

Resources Laboratories as the five major elements currently constituting the VO Program at FCRC. At the same time laboratory renovations were essentially completed as was the final recruitment of staff. Thus plans that were envisioned in last year's annual report as a result of the restructured program became effectively realized with all working program elements implemented virtually to full capacity.

Program Derivation, Review, and Evaluation: FCRC/VO Program goals and objectives are set forth by the Scientific Coordinator for Viral Oncology, in residence at Frederick, subject to approval of the Associate Director for Viral Oncology. Recommendations that have played a significant role in the ultimate determinations of the FCRC/VO Program have been provided in the past by the Virus Cancer Program Advisory Committee, and Virus Cancer Program Scientific Review Committees, all entirely composed of six to eight recognized outside experts; scientific peer reviews have been conducted annually by the latter. In-house recommendations have been obtained on an as needed basis from NCI staff, such as a number of NCI program monitors and/or other senior staff personnel who provided specialized individual or ad hoc committee assistance.

All work conducted within the VO Program at FCRC is reviewed on a continuing basis for the purpose of attaining and maintaining excellence of programs that have been established, and to insure rapid response of VO/FCRC operations to needs related to Viral Oncology and NCI Program objectives. It is the responsibility of the SCVO to oversee implementation of NCI Viral Oncology programming at FCRC and to facilitate and participate in a number of review and evaluative processes that are necessary to insure maximal qualitative and quantitative accomplishment.

The first line of review is the periodic evaluations by NCI Monitors of VO/FCRC work performed by the Contractor. The Monitors, one functioning for each of the Laboratories, were carefully selected on the basis of their scientific background and experience related to the work conducted. It is the responsibility of the NCI Monitors to discuss all aspects of the work on a monthly basis with the appropriate Contractor manager and to submit bi- or tri-monthly written evaluations on progress made and/or problems that have arisen during that period of time. The written evaluations are reviewed by the SCVO who provides these together with his own comments for transmittal to appropriate NCI authorities and to the Contractor for their consideration. Reviews conducted in this manner provide a rapid system of communication whereby the Contractor can be commended for progress in a given area and/or apprised of shortcomings in the work performed so that, in the event of the latter, these can be corrected without undue delays. In addition, the monthly evaluations are cumulatively judged semiannually, forming the basis

upon which the Contractor's award fee is determined. Based upon input from the SCVO, final fee recommendation is made by the Award Fee Board, consisting of senior NCI administrative staff members, for final approval by the Director of NCI.

This year for the first time a number of VO Laboratory operations have been placed under a different fee evaluation system. Formerly, all work conducted in the VO/FCRC program except for the VO Director's research area, involving effort in immunochemistry and immunobiology of oncornaviruses, was evaluated on an award fee basis. Under the present system, all contractor research effort in the RNA Virus, DNA Virus and the Immunochemistry Laboratories was exempted from award fee consideration; these were on a fixed fee. Remaining under the award fee system were the Viral Resources Laboratory and collaborative efforts involving NCI personnel in the Disease Control Laboratory (the total effort in this case), as well as those in the RNA Virus and DNA Virus Laboratories. Also under this system was the Contractor VO Director's administrative operation. This includes all Contractor VO planning, FCRC/VO administrative/clerical support, technical laboratory support, e.g., electron microscopy, product quality control, animal handling, media and glassware preparation and janitorial services.

General Program Consideration and Objectives: The overall objective of the Viral Oncology Program at the Frederick Cancer Research Center is to conduct investigations on oncogenic and suspected oncogenic viruses and their interactions with host cells to: (i) determine whether viruses comparable to those known to induce cancers of laboratory and domestic animals are associated with, indicative of, or, in fact instrumental either alone or as co-carcinogens in inducing the development of human cancers, (ii) define the character and mode of action of virus and/or viral components in their relationships to tumor formation, and (iii) to devise therapeutic and preventive measures for their control. Functional laboratory elements within the VO/FCRC program will conduct basic, exploratory and applied research on programs that are or could be vital to the Cancer Program and provide expert collaborative support for several on-site NCI investigators. Moreover, as a highly important resource activity, a substantial effort will be maintained for the purpose of providing critically needed working materials for the Virus Cancer Program both within and outside FCRC.

The Viral Oncology Program at FCRC inevitably undergoes changes each year in order to reflect new scientific developments, alterations in outside related activities, and in general to improve upon and or take maximal advantage of accomplishments within the current effort in implementing research supporting Viral Oncology and the National Cancer Institute's program of coordinated research on viruses as etiologic agents of cancer. Significant changes within the past two years included the restructuring of the VO/FCRC program into four and later, five Laboratory elements, and the appointment of the

Contractor's Director of Viral Oncology, which brought to fruition a desired redirection of the viral oncology effort. The five major elements constituting the framework within which all scientific effort in the VO/FCRC Program will be conducted are the following: Immunochemistry Laboratory, RNA Virus Laboratory, DNA Virus Laboratory, Disease Control Laboratory, and Viral Resources Laboratory. The first of these, representing the most recently added VO/FCRC program element, was formed for the purpose of furthering the development of basic investigations in cancer virology within such disciplines as biochemistry, molecular biology, immunology, cell biology and serology and to provide highly specialized technical augmentation in the form of collaborative operations with other existing VO/FCRC programs.

It should be emphasized again, as in past years, that the FCRC Viral Oncology effort is to be performed as a total integrated activity that will allow for a high degree of flexibility in response to overall program needs and for broad latitude in creating and pursuing fundamental research objectives both in-house and on a collaborative basis with outside acknowledged experts. As work in given areas is performed, NCI/VO may determine that particular research or resource goals require changes in emphasis; objectives may be altered and/or previously established ones postponed or eliminated. Conceptual modifications may be introduced, as for example the recent increased program interest in viruses and chemicals as co-carcinogens. Programs will continue to be reviewed periodically by NCI and/or selected outside peer reviewers and determinations made as to their need or desirability for continuation while new projects may be evaluated for potential program merit and exploratory effort.

More specifically, lines along which the FCRC/VO Program proceeded during the past year are as follows:

Immunochemistry Laboratory. Contractor staff in this Laboratory were responsible for the implementation of basic investigations, examples of which would include studies on the natural history of oncogenic viruses using immunological, virological and molecular biologic methodology. Utilizing specific viral or viral-induced products, attempts were made to interrupt viral, chemical, and spontaneous neoplastic processes. Potentially useful immunogens related to or influencing host or tumor cell responses, e.g., viral proteins and/or virus modified cell proteins, were purified, characterized, and assayed for biologic activity. Studies were performed on the immunobiology of reverse transcriptase containing viruses (retravirus)-host cell and virus-natural host relationships with the ultimate aim of intervention in the disease process in appropriate animal models. Such work involved, as examples, the isolation, identification and characterization of oncornavirus induced cell membrane antigens, development of assay systems for type C virus proteins, searches for expression of viral structural proteins in human materials upon development of suitable assay procedures, production and evaluation of rodent and primate antisera prepared against specific viral and/or tumor antigens, and searches for human antibody against known or newly isolated viral structural proteins. Expertise incumbent within this effort embraced all aspects of protein chemistry and immunobiology.

RNA Virus Laboratory. Research in this area was directed toward achieving objectives within several project elements designed to elucidate the potential role of RNA viruses as etiological agents in human cancer. Investigative research was conducted on host range, natural history, replication cycles, and molecular biology of both B and C types. Among studies to determine species distribution, attempts were made to detect related viruses and/or viral or viral-induced components in human materials. Some redirected effort was concerned with the development of methods for studying chemical and viral-chemical co-carcinogenesis.

In the Characterization of Type B Viruses Section the primary objective of this effort was to conduct a program of research designed to determine biological, biochemical, serological, and other characteristics possessed by type B viruses that are currently available in the VO/FCRC program. Comparative studies were performed to provide information to ascertain and identify properties that may either be shared or that are unique for a given virus.

Using materials obtained from the Viral Resources Laboratory (VRL), work continued on MMTV to more fully characterize viral components from the established Mm5mt/c_1 and/or other developed cell lines, such as major and minor proteins, 60-70S materials, and nucleic acid fractions for hybridization studies. Close collaboration was also established with VRL for evaluation of Mm5mt/c_1 (or other subclone products) and virus materials obtained under specific cultural conditions, e.g., in chemically defined media, etc. Experimental requirements gave rise to the need for further studies on additional MMTV isolates to enhance the overall understanding of type B virus characteristics. Attending these studies, some effort was continued on the comparative characterization of MPMV and squirrel monkey virus.

In the Characterization of Type C Viruses Section the main objective of this effort was to continue in establishing and conducting a research program to comprehensively characterize type C viruses available in the VO/FCRC program. Specific lines of research, similar to those in the already established type B virus effort, were pursued with the most promising leads selected for further work. Experimental components, uniquely synthesized cell products, and associations or implications that may be made among these with biological, virological and biochemical events occurring in the human neoplastic process were developed. Attending this is the application of new methodology for inducing human cells. This effort was performed in close collaboration with the Immunochemistry Laboratory.

The Contractor provided necessary collaborative support services to NCI investigators engaged in the examination of normal physiological functions that lead to the activation of endogenous type C viruses and to determine what, if any, role such activated viruses, in turn, play in normal host functions and tumor induction.

DNA Virus Laboratory. The Contractor engaged in a series of studies for the purpose of determining the carcinogenic or co-carcinogenic role of DNA viruses in the neoplastic process. Basic research was conducted with herpesviruses of human and non-human primate origin in vivo and in vitro systems to elucidate mechanisms of action in tumorigenesis and cell transformations. In collaboration with on-site NCI investigators, some effort was expended to design and/or perfect biological, serological, and biochemical techniques to identify significantly active (e.g., transforming or other) regions of herpesvirus DNA, to detect genomic fragments and/or proteins that might be found specifically in herpesvirions and/or transformed cells, to characterize factors released from tumor virus cells in vivo and/or in vitro as markers of oncogenesis, and to investigate the feasibility of using specific viral or viral-associated antigen reagents to search for evidence of herpesvirus information in human disease and to attempt to correlate their presence with the incidence of human cancer.

The Contractor provided necessary professional/technical collaboration and support services for senior NCI investigators presently involved in on-going comprehensive biological, serological and biochemical studies related to the role of herpesviruses as transforming agents and/or as carcinogens or co-carcinogens possibly in conjunction with type C viruses or chemicals, in tumor formation. Also included was the preparation, assay, and purification of various HSV strains and nucleic acid probes for testing transformed cells for residual HSV sequences.

Disease Control Laboratory. The Contractor continued to develop and maintain Disease Control Laboratory facilities for the purpose of conducting a multi-faceted VO/FCRC program designed to study a variety of genetic, immunological, and viral synergistic factors that may influence the course of tumor development. Work was performed in collaboration with NCI investigators, a number of whom are located at FCRC. The objective of studies performed was to provide pertinent information as to the causative role of viruses in cancer and to develop rational approaches to the prevention and cure of these diseases. Suitable laboratory facilities together with professional and technical staff that possess expertise in genetics, immunology, cell culture, biochemistry and animal technology were required. In performing the operations of this Laboratory, it was incumbent upon the Contractor to continue collaborative working relationships with other VO/FCRC program elements, as for example in viral resources, developmental research, and the Immunochemistry Laboratory, and also with NCI investigators who are engaged in studies of similar nature both at FCRC and outside laboratories.

Viral Resources Laboratory. The continued goals of work in this area were to conduct a major resource effort and provide facilities for the large-scale production of viruses or viral components that are necessary for performing laboratory research in the VO/FCRC or associated outside VO programs; to

engage developmental research on oncogenic or suspected oncogenic viruses necessary to the VO program but for which no previously established protocols exist or for which protocol improvement is required; to develop suitable quality control support activities involving electron microscopy, tests for contamination, and other appropriate biochemical, serological and biological tests to insure maximal quality of resources.

FCRC/Viral Oncology Administrative and Support Area. The Viral Oncology Program (VOP) at FCRC provided for the establishment of comprehensive basic, developmental, and applied research and resource efforts, which in totality constitute a highly flexible, multi-disciplinary and interrelated operation addressing the problem of determining the role(s) viruses, their activities, and their products play in the cause, detection, and ultimate prevention of neoplastic disease. Along with designated NCI authority, Contractor responsibility for the VOP was to oversee VO/FCRC operations in such a manner so as to insure that work conducted toward attaining NCI/VO program goals was performed with maximal effectiveness. The Contractor's Director of Viral Oncology (DVO) exercised main supervisory responsibility for the conduct of all VO/FCRC Laboratory activities described above, including Contractor personnel and facilities in collaborative support for on-site senior NCI investigators. Utilizing professional and technical staff expertise and facilities at his disposal, the DVO executed the deployment of these elements not only to optimally pursue VO objectives currently in existence but also to assist NCI authorities in identifying new or modified areas of research that could be of benefit to the VO/FCRC effort and the Virus Cancer Program in general.

Within the Contractor's VO/FCRC administrative area, there were several important ancillary staff, professional, and technical groups that provided necessary support for Laboratory operations and assisted the DVO in discharging his supervisory responsibilities.

The first of these identified is the administrative staff. This group furnished assistance in the day-to-day handling of the Contractor's VO operations, as required by the DVO and professional staff, including various administrative, coordinative, secretarial, and editorial duties.

The second provided two important professional support functions involving Electron Microscopy and Quality Control. The former furnished necessary cooperative assistance for a variety of investigational, resource and collaborative research activities conducted within the total VO program at FCRC. Particle counts, thin section preparations, and ultrastructural analyses are some general examples of the work performed by the electron microscopy staff.

The Quality Control Group essentially provided routine testing services for the isolation and identification of mycoplasmas from cell cultures and serum in support of the VO program at FCRC and, pending availability of facilities and personnel, to the Virus Cancer Program, and also provided a testing service with the Bureau of Biologics, FDA, via an interagency agreement.

A third category of activities consisted of technical support in the form of animal care, glassware preparation, and custodial services applied as required throughout the VO program.

Finally, it was recognized that, as the chief Contractor authority in the viral oncology area, the DVO had the responsibility for promulgating and maintaining adequate safety practices throughout the VO/FCRC Laboratory areas. This responsibility was shared on a designated basis with the various Laboratory section leaders to insure that an adequate safety awareness permeated all levels of VO personnel activities. In pursuing this endeavor, close cooperation was established and maintained with FCRC Biohazards and Environmental Control. Their advice and assistance was secured in matters dealing with but not necessarily limited to: (i) establishing safe operational procedures to insure protection of the worker and experimental integrity, (ii) periodic facility and equipment inspections, (iii) facility designs for new or renovated areas, (iv) proper disposal of waste materials, and (v) adherence to prescribed safety regulations.

Other ancillary objectives were as follows:

Environmental Control. To conduct a comprehensive safety and environmental control program for the Frederick Cancer Research Center and to perform applied and basic studies and literature surveys for risk assessment in support of the various FCRC operations.

Animal Breeding. To operate an animal farm for the breeding of laboratory animals to meet the needs of research programs at FCRC, and for shipment to other NCI operations as production permits.

Animal Health Diagnostic Section. To provide for monitoring the health and genetic quality of research animals at the FCRC and NCI in order to ensure the validity of animal-related research and to preclude the entry of undesirable pathogenic microbes, latent murine viruses and parasites.

Program Projections. The currently designed FCRC/VO Program inherently provides a strong but flexible scientific mechanism for providing the continued opportunity to enhance program productivity, by maximizing results of previous efforts as a basis for further work, for rapidly capitalizing on new scientific and technological advances, and for seizing new initiatives in previously untried areas of investigation as related to human cancer.

Plans will remain in effect to continue basic investigations studying the natural history of oncogenic viruses using immunological, virological and molecular biologic methodology. Utilizing specific characterized viral products, e.g., viral envelope proteins and viral induced cell surface antigens, attempts will be made to interrupt viral, chemical and spontaneous neoplasia in appropriate models. Products of tumor cell responses will be completely characterized and assayed for biologic activity. More specifically it is planned that work will proceed along the following lines: (a) Immunochemistry, for the isolation, characterization and antiserum production of

important viral or viral-associated proteins; (b) Molecular Biology, to prepare specific transcripts of RNA and DNA oncogenic viruses for studies of virus interrelationships, relationship of viruses to host genomes, transcription of viral genomes under experimental conditions, and preparation and analysis of transcripts specific for certain viral function; (c) Viral and Cell Biology, to develop assays for test viruses, optimal cell lines for virus expression and production, mechanism of virus induction, localization of viral genomes to specific chromosomes using somatic cell hybridization; (d) Primate (including man) herpesviruses, to study transformation by these viruses or their infectious DNAs, to compare biological activities among various strains, to analyze immunological factors that are conducive or deleterious to cell transformation and tumorigenesis, and (3) Virus, Reagent, and Cell Production to provide a large-scale central resource for working materials.

Finally, fruitful collaboration will continue with on-site NCI investigators engaged in ongoing studies on genetic and vaccine control of virus expression, the carcinogenic and/or co-carcinogenic role of herpesviruses in cell transformation and tumorigenesis, cellular enzymes involved in processes repressing or derepressing cell components, cell membrane property changes induced by virus infection, and the influence of host physiology on oncogenesis and cellular control.

SUMMARY REPORT

B. 2. a. LABORATORY OF DNA TUMOR VIRUSES

October 1, 1977 - September 30, 1978

The Laboratory of DNA Tumor Viruses plans and conducts research on viruses with a DNA core to define their role in the development of cancers in animals and man; develops and applies biological, biochemical and immunological procedures to obtain evidence for virus genetic expression in neoplastic cells; and investigates the mechanisms by which cellular gene expression, viral gene expression, and the interactions between viruses influence transformation of cells.

The Microbiology Section applies virologic, biochemical and immunological techniques to the study of the biology of herpesviruses and the nature of their association with certain cancers in humans and in animals with emphasis on the elucidation of the mechanisms whereby viruses of this group establish occult infections in cells which terminate in neoplasia; and defines the role of herpesviruses in oncogenesis by investigation of their effect on normal cellular control mechanisms and of the effects of herpesvirus and type C RNA virus interactions in dually infected cells. The Virus Tumor Biology Section studies the mechanisms of cellular transformation induced by human and simian papovaviruses; evaluates the effects of viral infection on cellular control mechanisms by comparing transcription and translation of virus genome information in transformation and in cytolytic responses to infection; and studies specific portions of the viral genetic sequences responsible for cellular transformation by genetic mapping of the small DNA viruses. The Virus Tumor Biochemistry Section conducts research on the specific protein and nucleic acid components of DNA viruses, and includes capability for the isolation and characterization of virion structural and nonstructural proteins of RNA viruses for collaborative studies on virus interactions in the oncogenic process; studies translational processes in vitro; and maintains electron microscope support for the research conducted by the laboratory. The Cell Physiology Section studies the alterations which occur in cells transformed by various means, including DNA tumor viuses; identifies enzymes of viral or cellular nature and determines the effects of transformation on cellular enzymes; investigates the influence of virus infection on the regulation of cellular enzymes with emphasis on the delineation of processes which result in repression or derepression of normal cell components; studies changes in the properties of cellular membranes induced by virus infection and their relationship to malignancy; and provides expertise for the study of both RNA and DNA viruses to complement activities within the Laboratory. The Primate Virus Section studies the relationships between virus and host factors that contribute to the pathologic processes involved in oncogenesis with particular emphasis on herpesviruses associated with induced neoplasia and cellular susceptibility genes that may predispose individuals to the development of cancer. The Office of the Chief coordinates the research conducted in the Sections with due recognition to the scientific freedom of the individual investigators. The Office is responsible for establishing collaborative research efforts between investigators in the Laboratory and other laboratories at NIH or elsewhere. The Chief directs the general activities within the Laboratory with the aid of the Assistant Chief.

The research activities of the Laboratory include investigations on DNA viruses in relation to: 1) transformation of cells and their conversion to malignancy; 2) genetic regulatory processes; and, 3) etiology of cancer in humans and animals.

Members of the herpesvirus group have been shown to induce malignant disease in animals and have long been suspected to be involved in the induction of some cancers in humans. The demonstration of transformation of hamster and mouse cells by ultraviolet-irradiated herpes simplex virus (uv-HSV) supported the hypothetical role of the type 1 and type 2 strains of this virus in the etiology of oral and genital human cancers, respectively. Previous work in this Laboratory demonstrated that uv-HSV activates the expression of an endogenous, xenotropic type-C RNA virus genome in mouse cells. The role of this virus in cell transformation is not known. However, its activation following herpesvirus infection suggests that these two viruses may be co-factors in the induction of transformation. Work during the past year was conducted to gain further insight into HSV-induced transformation.

Herpesviruses other than HSV were shown to activate a xenotropic type C virus genome endogenous in mouse cells. Ultraviolet irradiated pseudorabies virus, infectious bovine rhinotracheitis virus and a simian adenovirus, SA8, were effective activators. Furthermore, similar activity was demonstrated by isolated DNA prepared from HSV type 1 and 2, pseudorabies virus, infectious bovine rhinotracheitis virus, Simian SA8 virus, Epstein-Barr virus, human cytomegalovirus, Herpesvirus saimiri, Herpesvirus ateles, and Marek's disease herpesvirus. Fragmented HSV DNA of 3×10^6 daltons average size was effective; smaller fragments, averaging 1×10^6 daltons, were ineffectual suggesting a limiting size for the activating "gene". No activating properties were exhibited by DNAs extracted from vaccinia virus, SV40, animal cells, bacterial cells, and mycoplasma. It appears that the ability to activate endogenous type C virus gene expression in mouse cells is a unique attribute of the herpesviruses as a group. There was no evidence that these viruses activated an endogenous viral genome expression in human cells.

The transforming activity of uv-HSV was compared with that of SV40. Three increasing stages of cell transformation were defined in 10E2 cells, a subline of the BLP line of nontransformed, nontumorigenic BALB/c mouse cells: (1) alterations in cell morphology and cell growth properties; (2) cell growth in agarose-containing medium but not in agar-containing medium; (3) cell growth in agar medium and tumorigenic properties in vivo. Both uv-HSV and SV40 readily induced stage 1 transformation of 10E2 cells. HSV-induced transformants failed to reach the second stage unless the 10E2 cells had undergone more than 30 serial passages in vitro before infection. No uv-HSV induced transformants reached the third stage. SV40 showed little tendency to induce third stage transformation unless the cells were of high passage in culture when infected. Even then, less than 0.1% of the transformed cells exhibited properties of malignancy. Similar results were obtained when these tests were repeated with SV40 and HSV DNAs.

The region of the herpesvirus genome associated with cellular transformation has not been characterized. With other DNA tumor viruses, the region of the genome expressing early gene products is required for morphological transformation. While these regions have been delineated in herpesviruses, many of the immediate early alpha-polypeptides are mapped in the short unique region and in the adjacent terminal repetition. It has been demonstrated that this region gives rise to defective DNA (dDNA) in the Patton strain of HSV-1 suggesting that this segment also contains an origin of replication. Passage of HSV at high multiplicities of infection causes the synthesis of virions containing dDNA of increased density and reduced complexity by restriction enzyme analysis when compared with nondefective DNA. The origin of dDNA was physically mapped in the right hand terminal repeat region of the viral genome extending into only one side of the short unique region. The dDNA was of heterogeneous size (5.3 to 5.7 megadaltons), probably the result of variable termination in the small unique region. The dDNA together with its inherent heterogeneity was first observed at the fourth virus passage. The same dDNA size classes were still observed at the twentieth passage with no apparent selective loss of this heterogeneity. Cells infected with defective HSV-1 were examined for DNA binding proteins, and RNA extracted from these cells was translated in a cell-free system. A 20,000 dalton polypeptide extracted from the cells preferentially bound to double-stranded DNA. The RNA extract, when translated, showed overproduction of the same size polypeptide, presumably specified by the dDNA. Passage of HSV-2 strain 333 at high multiplicities of infection also results in the appearance of virions containing dDNA with increased density and decrease in complexity. Both HSV-1 and HSV-2 dDNA have been shown to be related by hybridization suggesting that the same regions of both viruses give rise to defective genomes.

As the initial step in elucidation of the enzymes associated with herpesvirus replication, the DNA dependent DNA polymerases from HSV-1 and herpesvirus saimiri (HVS) have been purified, and their activities have been compared to the normal cellular DNA polymerases. Both viral polymerases are stimulated by high salt and exhibit normal activity with an oligomer-homopolymer consisting of deoxyguanylate and deoxycytidylate. This preference may be related to the generation of the high G-C dDNA of HVS originating from the ends of the HVS genome.

Comparative studies on Moloney murine sarcoma virus (MSV) suggest that a large class of defective MSV molecules exists in the uncloned MSV stock. The gag gene polyproteins expressed by each of five clonal isolates of MSV stock were found to be unique in size, ranging from 60,000 to 70,000 daltons. The polyproteins specified by two of the isolates lack p10 indicating that these MSVs have deletions in their 5' p10 gag region. These polyproteins are the largest products synthesized both in cells and in cell-free translation systems. The variable size of the polyproteins expressed by each defective MSV isolate suggests heterogeneity in the size of this gag gene deletion and may be similar to the size range that occurs in the dDNA of HSV-1.

The gag polyproteins of two MSV isolates, m1MSV P60 and m3MSV P70, were characterized. The gag gene order was shown to be N-p15-p12-p30-C. Both polyproteins had similar peptide maps from the N-terminus through two-thirds of p30, both lacked p10 and both had different C-terminal peptides. While both m3MSV and Moloney leukemia virus p30 had identical peptide maps, unique peptides were detected in the C-terminal 10 K portion of m1MSV p30, suggesting that the gag gene deletion in m1MSV may begin in the p30 reading frame. In addition to the differences in the size of the gag polyproteins expressed by the two MSV isolates, differences in their biological reversion frequency may indicate a relationship between the gag polyprotein expressed and the stability of the transformed state.

The BK virus and the JC virus, papovaviruses for which humans are the natural host, can produce tumors in hamsters. In this Laboratory, two types of highly sensitive nucleic acid hybridization tests, reassociation kinetics and the Southern blotting technique were applied to determine any association between the BK virus and cancers of humans. No evidence of significant homology beyond the "background" levels in controls was detected between the nucleotide sequences of BK virus and human tumors or continuous lines of human, tumor-derived cells. Similarly, indirect immunofluorescence tests showed no positive finding of SV40, BKV or JCV reactive T-antigen or T-antibody in the specimens from cancer patients examined. These results suggest no significant association between cancer in humans and evidence of infection by these papovaviruses.

A principal undertaking in our Laboratory has been investigation of SV40 mRNAs synthesized during a lytic infection in permissive monkey kidney cells. RNA transcribed from DNA in cell nuclei is found in the cell cytoplasm in a modified, polyadenylated form. This processing of messenger RNA was studied in African green monkey kidney cells infected with SV40. The late 16S and 19S viral mRNA transcripts found in the cell cytoplasm were mapped. It was found that a leader of about 210 nucleotides transcribed from one region of the virus genome (0.72-0.76 map units) is spliced to the main coding sequences of 16S mRNA transcribed from a different region of the SV40 genome (0.94-0.17 map units). Sequences present in the viral genome which lie between these map positions are absent in the cytoplasmic message. Examination of the viral cytoplasmic 19S mRNA identified the genomic map positions of several sequences of nucleotides which are represented as leaders spliced to the same coding sequences of the body of the message (0.765-0.17 map units). A large fraction of cell nuclear RNA consists of molecules containing the sequences intervening between leader and body which apparently are deleted during processing of transcripts. Nuclear RNA transcripts also are found which extend beyond the terminus of polyadenylated cytoplasmic RNA at 0.17 map units; termination of transcripts at 0.17 map units appears to be associated with polyadenylation.

Investigations were initiated to determine the role of posttranscriptional degradative processing in the control of gene expression in cells. Monkey kidney cells were infected with wild-type SV40 or a variant of the virus containing reiterated and unique sequences of host-substituted DNA. Virus transcriptional complexes extracted from the cell nuclei produced transcripts in vitro. These RNAs were compared in amount with the wild-type or host-substituted RNAs extracted from intact cells. Much less host-substituted

sequences were represented in the RNA from intact cells as determined by a hybridization procedure. This suggests that the host sequences were transcribed but rapidly degraded in the intact cells. The alternative, artificially enhanced transcription of host-substituted sequences in vitro, could not be rigorously excluded, and further study is required.

Two phases of gene expression occur during the cycle of SV40 replication in permissive cells. An early phase of transcription which takes place prior to viral DNA replication is characterized by the synthesis of RNA coding for the production of T-antigen. In the presence of a functional T-antigen, a late phase of transcription produces large quantities of RNA coding for viral structural proteins. Investigations were initiated to clarify the viral and cellular functions responsible for the transition from the early to the late phase of the cycle. It was found that a substantial fraction of the early RNA is transcribed from free rather than integrated viral templates. Two separable peaks of activity, revealed by sedimentation analysis of early viral transcriptional complexes, however, suggest the existence of two distinct types of early SV40 templates. It was shown that small amounts of late SV40 RNA can be synthesized prior to DNA replication. This indicates that the transition to predominant synthesis of late RNA occurring after DNA replication is a quantitative phenomenon. Further study is projected to elucidate the role of functional T-antigen in the regulation of synthesis of viral mRNAs.

SV40 tsA mutants which make a non-functional T-antigen at the nonpermissive temperature were selected to investigate the function of the early gene products in lytic and transforming infections. At the nonpermissive temperature, the tsA mutants were found to overproduce all the early transcripts and all known early viral proteins in cells lytically-infected as well as in cells transformed by the mutants. One of the early proteins appeared to be preferentially produced in hamster embryo cells transformed by a tsA mutant, suggesting either preferential transcription or processing of the RNA for this protein. We are investigating potential differences in the splicing pattern of early transcripts in cells infected with the tsA mutants held at permissive and nonpermissive temperatures. Such mechanisms may operate to maintain a viable lytic or transforming infection.

The early events in transformation appear to involve an abortive infection of nonpermissive cells. Mouse embryo cells are nonpermissive for SV40. Following infection, early gene protein products are expressed but late virus-specified antigens do not appear. Examination of mouse embryo cells following infection by SV40 showed that both early and late SV40 genes are efficiently transcribed at a ratio of about 60:40, respectively. Transcription peaks at about 10 hr and thereafter drops sharply to low levels. Late gene transcripts are detected in nucleus, cytoplasm and in association with polysomes, but do not appear to be translated at detectable levels. The nature of this potential block in translation is being investigated. It is possible that an alteration in transcription or posttranscriptional processing might block the translation of the RNA into the appropriate polypeptides. In addition to our investigation of the acute infections of permissive and nonpermissive cells, we have also examined stable transformants of SV40 and BKV in which the viral genomes are covalently integrated within the host

cellular DNA. We are using hybridization technology in an attempt to define the sites of integration in transformed cell lines. By comparing these to "cured cell lines" which still retain certain viral DNA sequences, but have lost the ability to express viral antigens as well as certain of the characteristics of the transformed phenotype, we hope to learn something about both transformation and the mechanisms for integration and excision of foreign DNA.

To obtain a more basic understanding of these processes, the mechanisms of integration and excision of a viral genome into and from a host chromosome were studied using lambda virus. In selected virus mutants, it was shown that the viral integrase enzyme contains discrete sites for the integration and excision functions. Integration of one DNA molecule into another occurs at specific attachment sites. However, an integrase-promoted recombination between two mutant lambda virus genomes demonstrated that recombination also occurs at low frequency in regions close to, but outside of, the attachment site. By insertion into a small plasmid, the genes for integration and excision could be studied in the absence of all other virus gene expression. No gene product for excision is made in such plasmids. Addition of cII and cIII gene products is required to achieve high levels of integrase function. Hybrid plasmids were constructed that expressed integrase constitutively in the absence of the cII and cIII products. In these hybrids, the integrase expression depends on promoters contained within the lambda DNA fragment.

A development of the technology associated with prokaryotic systems will also prove invaluable for future studies we intend to perform using recombinant DNA molecules. A major committment has been made to clone different classes of defective virus molecules in bacterial vectors so that the problem of rigorous comparison of their heterogeneity can be addressed. This technique will also allow the determination of viral gene functions and products that are associated with defined portions of the viral genome.

retroviruses

SUMMARY REPORT

B. 2. b. LABORATORY OF RNA TUMOR VIRUSES

October 1, 1977 - September 30, 1978

The major goals of the Laboratory of RNA Tumor Viruses are to determine the etiology of naturally occurring cancers and to develop experimental strategies capable of prevention of spontaneous and virus-induced tumors. The ultimate aim of these investigations is to apply approaches successful in animal model systems to the identification of causative agents of human malignancies and to the prevention of cancer in man. The primary emphasis of many ongoing investigations within the Laboratory concerns RNA tumor viruses. These viruses are unique among animal viruses in their mode of transmission and the intimate association that has evolved between these agents and cells of a large number and wide variety of vertebrate species. The etiologic role of this virus group has been established for naturally occurring cancers of many species, including some subhuman primates. Certain members of this virus group, so-called "replication-defective" transforming viruses, appear to have arisen by a mechanism involving recombination with cellular transforming genes. As such, these viruses offer an unparalleled opportunity to elucidate the processes by which such genes cause malignancies.

* The majority of RNA tumor viruses (retroviruses) are designated as type-C on the basis of morphological features. Retroviruses that cannot be classified as type-C include the bovine and guinea pig leukemia viruses, as well as a new group of primate retroviruses, designated type-D. LRTV scientists are actively exploring these viruses to determine their mechanisms of transmission and their roles in disease. Thus, research within the LRTV encompasses efforts to understand the basic processes in tumorigenesis, utilizing RNA tumor viruses as models. At the same time, studies are in progress to develop and apply the most sensitive and specific methods of tumor virus detection to the search for related oncogenic viruses of man. Major research efforts of the LRTV in these areas are centered in the Sections of Molecular Biology, Viral Genetics, and Field Studies and Epidemiology.

Investigations in the Laboratory over the past several years have suggested that prevention of spontaneously and chemically induced cancers in animal model systems might be accomplished by active or passive immunization with appropriate preparations specific for tumor cell antigens held in common by various neoplasms of a given species. Efforts have focused on the determination of which endogenous viral gene products might be the most effective immunogens, utilizing tumor prevention as the end point. Achievement of this goal not only fulfills the pragmatic objectives of cancer prevention but at the same time may provide important insights into mechanisms involved in the causation of cancer. These efforts have broadened in scope to include the search for shared tumor antigens that may be coded by endogenous transforming (src) genes. The Viral Immunology Section has the major responsibility within the LRTV for research in these areas.

In the past year, LRTV efforts to elucidate mechanisms of carcinogenesis have been significantly complemented and enhanced by the addition of the In Vitro Carcinogenesis Section. Research of this Section is aimed at determining mechanisms of spontaneous and carcinogen-induced malignant transformation of cultured cells of rodent and human origin. Special emphasis is being directed at the development of culture systems utilizing human epithelial cells to study the interactions of chemical carcinogens and viruses with cellular DNA. Further efforts are aimed at defining the fundamental cytologic, biologic, and biochemical characteristics of carcinogenic change.

The Virus and Disease Modification and Viral Immunotherapy Sections of the LRTV have as their major goals the development of effective modalities for prevention and/or control of virus-induced and spontaneously occurring neoplasia. These include the use of immunoadjuvants as well as chemotherapy and radiotherapy in well-characterized animal tumor systems. The eventual goal of these studies is the establishment of therapeutic approaches applicable to human disease.

During the past year, significant progress has been made by LRTV scientists studying the actions, distribution, mode of transmission, and pathogenesis of several major groups of RNA tumor viruses. Certain replication-competent type-C RNA viruses are known to be transmitted as oncogenic agents in animals. These include leukemia viruses of the cat and gibbon ape. Highly sensitive and specific radioimmunological techniques have been developed to detect exposure to such agents in the animal populations in which they are naturally transmitted. Because their animal reservoirs are maintained in close proximity to man, such viruses represent significant potential public health hazards. Extensive seroepidemiologic investigations have accordingly been conducted to assess the risk of human infection. In the cat, for example, recent studies have shown that as many as 10% of randomly tested cats demonstrate serologically detectable FeLV antibodies or antigens. In contrast, a large series of clinically diseased humans, including many with lymphoma or leukemia, were tested and shown to have no immunologic evidence of FeLV exposure. Similarly, sera from a large number of persons with occupational or home exposure to leukemic, FeLV-positive cats, from laboratory workers involved in RNA tumor virus research, and from normal controls demonstrated no immunologic evidence of FeLV infection. These findings do not exclude the possibility that FeLV may be transmitted to individuals with unusual exposure or altered host defenses; thus, continued screening of such persons for evidence of infection remains important. However, it can be concluded on the basis of these findings that FeLV infection occurs rarely, if at all, in the human population so far studied, and is unlikely to be a significant cause of disease in man. Analogous studies are currently being performed to assess the likelihood of human exposure to other known oncogenic retroviruses.

In the past several years, work by this Laboratory, as well as others, has led to the understanding that type-C viruses, analogous to those known to be causative of cancer, are genetically transmitted as endogenous viruses within many vertebrate species. Major questions concerning these viruses include their biologic function and whether or not they exist in man. Efforts at discerning biologic functions of endogenous type-C viruses have

focused on the mouse model, primarily because of the power of genetic techniques available in this system. Previous studies from this Laboratory have shown that endogenous viruses of mouse strains, which are characterized by high incidence of leukemia, induce leukemia when inoculated into susceptible mice of a low leukemia incidence strain. These findings helped to establish that endogenous viruses can be oncogenic. In the past year, investigations within the Laboratory have shown that a type-C retrovirus, isolated from a mouse strain characterized by low leukemia incidence, is very closely related to a known endogenous virus of that strain. This B-tropic leukemia virus obtained from old-age BALB/c mice cannot be directly induced from BALB/c cells in tissue culture. By molecular hybridization and radioimmunological analysis, the B-tropic virus was shown to be very closely related to a known N-tropic endogenous virus of the same mouse strain. Moreover, genetic information of the B-tropic virus was demonstrated to segregate with a single structural locus for the N-tropic virus. This evidence supports the hypothesis that the B-tropic virus is either very closely linked to, or arises by a small genetic alteration of, the N-tropic endogenous virus. Such findings demonstrate the importance of genetic controls over endogenous retrovirus expression, since the lack of Fv-1 cellular restriction to B-tropic virus replication in the BALB/c mouse leads to its unrestricted growth and the development of neoplasia in this strain.

Several new endogenous type-C viruses have been isolated and characterized within the past year. These include a B-tropic virus inducible from the SWR inbred mouse strain. This virus was shown to be distinguishable by molecular hybridization from previous endogenous mouse type-C virus isolates. Moreover, nucleotide sequences specific to this virus were demonstrated to be present only within cellular DNA of the SWR, as opposed to other, inbred mouse strains. The inducibility locus for the SWR virus was shown to segregate with the viral structural information in appropriate genetic crosses.

A new, endogenous type-C virus of carnivores has also been isolated and characterized. Cells of the established MvlLu mink line were found to spontaneously release a reverse transcriptase-containing virus after long term passage in tissue culture. By molecular hybridization, DNA of normal mink cells was found to possess extensive nucleotide sequence homology with the reverse transcription product of this viral genome. The mink endogenous virus was shown to share antigenic determinants with the major structural proteins of known mammalian type-C viruses, as well as to possess antigenic determinants unique from those of other known retroviruses. By immunologic criteria, the mink endogenous virus most closely resembles FeLV and the endogenous type-C virus of the rat.

By molecular hybridization, the distribution of nucleotide sequences related to another recent endogenous type C virus isolate, this one of the Columbian black-tailed deer, O. hemionus, led to the demonstration of related genetic information in a wide variety of ungulates, including those of both New and Old World origin. This evidence indicates that endogenous viruses have been genetically transmitted within the ungulate family for several million years.

Studies within the Laboratory have led to the development of immunoassays for each of the known translational products of the mammalian type-C virus genome. These included p30, p15, p12, and p10 (encoded as part of the polyprotein precursor of the gag gene), reverse transcriptase (pol gene) and the envelope glycoprotein, gp70 (env gene). The virus-coded nature of each protein, as well as its unique antigenic determinants, has now been established for analogous proteins of a large number of mammalian type-C viruses. In the broadest interspecies immunoassays for each of these viral products, it has been possible to demonstrate that interspecies antigenic determinants are shared between p30, p15, p10 and reverse transcriptase, respectively, of all known mammalian type-C viruses. These findings strongly argue for a common progenitor in the evolution of this virus group.

A basic understanding of the mechanisms by which type-C RNA viruses induce cancer may require a detailed knowledge of the order of genes coded for by these viruses. Efforts within the LRTV have led to establishment of the ordering of viral proteins coded within the murine type-C virus gag gene as p15-p12-p30-p10. In collaborative studies, the amino acid composition of Rauscher and AKR murine leukemia virus gag gene-coded proteins has been determined. Moreover, the amino and carboxy terminal amino acids have been identified and sequenced. Both p15 proteins were found to have a blocked amino acid. The data obtained in these studies along with the previously established order of the type-C viral gag gene have permitted a definition of the cleavage sites and the enzymatic mechanisms involved in the post-translational processing of the gag gene coded-precursor polypeptide.

A strategy based upon the identification of type-specific antigenic determinants of the translational products of the type-C virus genome has been used to study genetic recombination between mouse type-C RNA viruses. By this approach, recombinants involving exogenous and endogenous type-C viruses have been identified. These techniques have also been applied to the investigation of genetic relationships between different endogenous viruses that exist within the same mouse cells. Proteins of the inducible class of xenotropic virus were shown to exhibit extensive antigenic homology with the gag but not the env gene products of the ecotropic virus class. The env gene coded glycoproteins of the inducible xenotropic virus classes possess striking antigenic homologies with the analogous proteins of the noninducible endogenous xenotropic virus. These findings support the concept that the inducible, xenotropic virus of mouse cells arises by a recombinational mechanism involving progenitors of two other endogenous viruses. Recombination between endogenous viral genes, thus, provides a mechanism for gene amplification in eukaryotic cells. By appropriate immunologic and genetic analysis of recombinant viruses obtained in crosses between leukemogenic and nonleukemogenic parental mouse virus strains, it may now be possible to identify and localize a region of the replication-competent type-C viral genome that is responsible for malignant transformation of lymphoid cells in vivo.

Investigations by LRTV scientists have focused on replication-defective mammalian transforming viruses. These viruses have been isolated from a variety of mammalian species including primates. During the last year,

efforts have continued to analyze translational products of defective transforming virus genomes. Cells nonproductively transformed by feline sarcoma virus were shown to express the FeLV gag gene-coded proteins, p15 and p12, but not other known FeLV-coded gene products. Like several previous transforming viruses characterized to date, the helper viral proteins expressed in feline sarcoma virus (FeSV) transformed cells were shown to be translated initially in the form of a high molecular weight precursor. The size of this precursor was approximately 125,000 daltons, much larger than could be accounted for by the helper viral proteins contained within it. Collaborative studies have further indicated that the high molecular weight precursor contains antigenic reactivity of the feline oncornavirus membrane associated antigen (FOCMA). This antigen has been reported to be present in naturally occurring feline leukemias and sarcomas, whether or not they are virus productive. If FOCMA is a product of the FeSV genome, it should make feasible efforts to purify and further characterize this protein. Essex and coworkers have presented substantial evidence to indicate that antibody to FOCMA is predictive of resistance to the development of cat lymphoma. Thus, the possibility that this antigen may be more readily isolated could be of great benefit in tests of the immunoprevention of FeLV-FeSV induced cancers. If, as now seems to be the case, FOCMA is a transforming virus-specific protein, it will be additionally important to determine whether FOCMA is, in fact, a product of the transforming region of the FeSV genome (src gene), and its role in viral transformation.

Using analogous techniques, cells nonproductively transformed by the replication-defective Abelson lymphosarcoma virus have been shown to express an 85,000 to 100,000 molecular weight polypeptide that contains the MuLV gag gene proteins p15 and p12 covalently linked to an as yet unidentified 60,000 molecular weight protein. The latter protein lacked antigenic reactivity with other MuLV-coded structural components. By analogy to data concerning FeSV, it will be of importance to further purify and characterize this transforming virus-specific protein.

In other studies, the RNAs of replication-defective murine and primate transforming viruses were analyzed for the presence of nucleotide sequences homologous to the genomes of their respective helper type-C viruses utilizing DNA complementary to either the 5' terminal or total nucleotide sequences of the helper virus RNA. The defective viruses examined were previously shown to vary in their ability to express helper viral gag gene proteins. With total cDNA as probe these transforming viruses were shown to vary in their representation of helper virus sequences (15-60% hybridization). In striking contrast, 5' terminal sequences of the helper virus were found to be conserved in the RNA of every sarcoma virus tested (over 80% hybridization of 5' cDNA). These findings indicate that a general property of mammalian transforming type-C viruses is their conservation of helper virus 5' terminal nucleotide sequences. This suggests a critical role of these sequences in the life cycle of the defective transforming virus. The continued application of molecular, biological, and immunological techniques to the study of transforming mammalian viruses is expected to yield critical information with regard to the organization of these viral genomes and the mechanisms by which the translational products of these viruses induce the striking and rapid alteration of cells both in tissue culture and in vivo.

Among those retroviruses that are not classified as type-C RNA viruses, LRTV scientists have focused efforts in the past year on the group designated type-D. The prototype virus of this group was isolated several years ago from a breast tumor of a rhesus monkey. In the past year, work by other laboratories has led to the isolation of similar viruses from New and Old World primates. Research within LRTV has helped to establish the endogenous nature of type-D retroviruses. The 35,000 dalton major structural protein (p35) of the squirrel monkey retrovirus (SMRV) was purified and shown to possess antigenic determinants distinct from those of other known retroviruses. However, in more broadly reactive immunoassays, antigenic cross reactivity was demonstrated between SMRV p35, and the major structural protein (p26) of the prototype type-D virus, Mason-Pfizer monkey virus (MPMV). These findings support the concept that SMRV and MPMV are evolutionarily related and raise the possibility that a progenitor of type-D retroviruses became genetically associated with primates at an early time in their evolution. The development of interspecies immunoassays for the detection of these primate type-D viruses may provide important probes for detection of related viral information in humans.

Further investigations have shown that the endogenous retrovirus of the guinea pig (GPV) is distinct from other known type-C RNA tumor viruses but shares certain morphological and biochemical properties with type-D viruses. A highly sensitive radioimmunoassay has been developed for detection of the major internal structural protein of GPV in order to study GPV expression in normal and malignant guinea pig cells, and to better determine the relatedness of GPV to other mammalian retroviruses. By competition radioimmunoassay GPV has been found to be distinct from any known mammalian retrovirus. Efforts have been initiated to develop in vitro conditions for cultivation of bone marrow cells to permit tests of the malignant potential of this agent in tissue culture.

Significant advances have been made in LRTV research concerning immunoprevention of spontaneous and virus-induced cancer. Protection against leukemia in the high leukemia incidence AKR mouse strain was achieved by passive immunization with antibodies against type-C viruses. Highly significant prevention of 3-methylcholanthrene-induced sarcomas was accomplished with passive transfer of IgG directed against the radiation leukemia virus. Analysis of antisera reactivities indicated that only those which possessed high titers against both ecotropic and xenotropic viruses offered significant protection, while sera with relatively low titers against xenotropic viruses offered little protection. In other studies, it was possible to demonstrate a reduced incidence of radiation-induced mouse leukemia after passive or active immunization against mouse type-C viruses. Additional evidence was obtained that active immunity can be induced in BALB/c and C57/Bl mice by use of an inactivated type-C virus vaccine. After vaccination, suppression or irradication of expression of the endogenous ecotropic virus was observed in spleens from these animals. Appropriate immunization regimens also led to a significant reduction in expression of the inducible xenotropic virus in the same strains. All of these findings indicate that immunoprophylaxis may be a feasible approach toward prevention of tumors caused by endogenous viruses.

In a rat tumor transplantation system, both syngeneic and allogeneic Kirsten sarcoma virus tumor cell vaccines were shown to provide transplantation immunity to a variety of lethal syngeneic rat tumors (sarcoma, carcinoma, and lymphoma) induced by chemicals, DNA tumor viruses, and by aging. These results support the concept that while KiMSV-coded translational products, directly or through their induction of other cellular genetic sequences, provide conditions that can lead to the development of cancer, these can be utilized in cancer prevention as well. Efforts are now underway to further define the effects of the most potent virus vaccines on spontaneous and chemically induced tumors in several different inbred murine strains. Efforts will also be undertaken to establish appropriate test systems for immunoprevention of cancer in subhuman primate models as a prologue to similar studies in man.

Over the past several years, scientists within the In Vitro Carcinogenesis Section, LRTV, have been involved in studies to elucidate the mechanisms involved in spontaneous malignant transformation of mouse fibroblasts in tissue culture. In the past year, studies have focused on the effects of light-induced chromosome damage on such cells. It has been shown that fluorescent light produces chromatin breaks and exchanges as well as DNA cross linkage. This damage can be minimized by lowering the oxygen tension of the gas phase or by addition of reducing agents such as glutamine to the medium. Thus, it can be inferred that hydrogen peroxide produced in the medium or cells is an important causative agent of these effects. Repeated exposure to light has been shown to accelerate neoplastic transformation in mouse cell cultures. The relative susceptibility of rodent and human cells to these types of genetic damage and a comparison of their respective repair capabilities may provide some clue as to the different susceptibilities of these cells to malignant transformation in culture.

Efforts have also been undertaken to develop improved culture techniques for growth of human epithelial cells in vitro. It is now possible to prolong subculture of human epithelial cells for several passages. To date, attempts to transform such cells with a number of chemical carcinogens have not been successful. However, continued efforts in this area are projected.

Studies to identify and quantitate cytologic and other characteristics associated with neoplastic transformation have continued. The most consistent morphologic features associated with neoplastic transformation have been (a) decrease in projected area of the cytoplasm; (b) decrease in projected area of the nucleus; and (c) decrease in the dry mass of the cytoplasm. In collaborative studies, the organization of microtubules and actin filament impaired non-neoplastic and spontaneously transformed rodent cell lines has been investigated. Similarly, scanning electron microscopy (SEM) has been applied to the comparison of cells of each type. Cytologic criteria found to be of diagnostic value in rodent systems will be further quantitated and applied to the analysis of transformed human cell cultures as these become available.

LRTV scientists have been actively involved in studies aimed at treatment of cancers in several rodent model systems. A variety of approaches have been utilized. In particular, several agents have been found to be effective immunoadjuvants to chemotherapy of an established murine leukemia and adenocarcinoma. These include Brucella abortus cell wall, glucan, pyran copolymer, and thymosin. Each was shown to increase the life span and cure rate of animals when these agents were used in conjunction with chemotherapy. Certain of these agents were found to stimulate macrophages in vitro and to exert a tumoricidal effect. Such agents were also effective adjuvants to tumor cell vaccines, strongly potentiating the immune response to live tumor cell challenge. Efforts to further elucidate the mechanisms of action of these agents may make it possible to devise even more potent immunoadjuvants and make possible clinical trials of their efficacy in humans.

A spontaneous mammary carcinoma in the guinea pig has been developed as a model for therapy of this disease. The tumor is a transplantable, slowly growing neoplasm which eventually metastasizes to the lung. Preliminary studies aimed at immunotherapy have not as yet led to reduction in metastatic disease. Further investigations will utilize a variety of approaches including chemotherapy, surgery, immune stimulation, and vaccines in this tumor system, as well as in the guinea pig leukemia system.

In addition to their intramural research efforts, investigators within LRTV serve on the editorial boards of major journals in their fields, serve as members of various grant and contract review bodies, and participate in a large number of collaborative efforts with scientists in laboratories throughout the country. The major goal of studies within the Laboratory is to apply, wherever possible, basic information derived from a multi-disciplinary approach to the study of virus-induced and spontaneously occurring cancers to its most important application, understanding of the cause and prevention of cancer in man.

SUMMARY REPORT

B. 2. c. LABORATORY OF TUMOR VIRUS GENETICS

October 1, 1977 - September 30, 1978

The Laboratory of Tumor Virus Genetics has focused its efforts in the past year on two major projects. One is a continuation and extension of work conducted in the laboratory over the past three years. This project involves the identification of proteins responsible for the transformation of normal to malignant cells and the identification of the functions of these proteins. The second project involves the application of recombinant DNA technology to RNA tumor virology in an attempt to elucidate better the structure and function of the genomes of a variety of RNA tumor viruses. The major work on the mechanisms of malignant transformation is conducted in the Molecular Virology Section, Cellular Transformation Section, and Carcinogenesis Regulation Section of the Laboratory of Tumor Virus Genetics. Two systems are being investigated to delineate the etiology of neoplasia. One system concerns the study of viruses that are capable of transforming undifferentiated fibroblasts in cell culture and utilizes the Kirsten and Harvey strains of murine sarcoma virus, and the Bryan strain of Rous sarcoma virus. The other system involves a virus which is able to produce rapid erythroleukemia in hematopoietic cells in vivo, and utilizes the Friend strain of murine leukemia virus. These two systems offer complementary approaches to identifying molecules responsible for the malignant transformation of undifferentiated cells and the transformation of differentiated hematopoietic cells to leukemic blood cells.

The major findings in the projects over the past year have provided important insights into both the origin of the transforming potential of these viruses and the identification, for the first time, of gene products responsible for the malignant effect. Work from this laboratory has shown that the Kirsten and Harvey viruses are recombinants between mouse and rat type-C viruses. A molecular and genetic map of the Kirsten and Harvey viruses has been constructed over the past year which provides new important information on their genesis. The 3' end of each of these viruses has been shown to be derived from the parental mouse leukemia virus. It is comprised of approximately 1 kilobase of mouse genetic information. The rest of the Ha-MuSV and Ki-MuSV genomes, ranging from 5 to 7 kilobases in length, are comprised of rat genetic sequences, and the 5' end of each virus is composed of approximately 100 bases of mouse type-C information. The genetic composition of these viruses indicates that they code for no structural proteins of known mammalian retroviruses. Identification of proteins coded for by these viruses may require different approaches from those employed to isolate and characterize structural proteins of previously identified retroviruses. Two proteins have been recognized in the last year which have been shown to be coded for by the Harvey and Kirsten viruses: a 22,000 dalton protein coded for by the 5' end of the Harvey genome, and a 50,000 dalton protein at the 5' end of the Kirsten

genome. The origin of the p22 is unclear but evidence has been accululated that the 50,000 dalton protein is coded for by the Ki-MuSV rat genetic sequences. Importantly, the p50 seems to represent the authentic protein that these rat sequences synthesize when they are expressed in naturally occurring rat tumors as well. For example, in studies on DMBA-induced rat breast tumors, high levels of expression of the Ki-MSV related rat genetic sequences have been found including that portion of the rat viral genome that codes for the 50,000 dalton KiSV-specific protein. Therefore, assays for the 50,000 dalton protein should be useful in the diagnostic evaluation of chemical carcinogen-induced tumors in rats and can provide new, sensitive, and rapid assays for chemical carcinogens in this widely used rodent testing system. Attempts are underway to develop immunoassays for the p50 or in situ hybridization for that portion of the rat genome coding for this 50,000 dalton protein. The function of the protein is not yet known.

In the Rous sarcoma virus system, a protein of 55,000 daltons in molecular weight, has been identified as the product of the sarc gene of RSV. This work has also been carried out in two other laboratories in the field of RNA tumor virology and this set of observations from these three laboratories strongly indicates that this protein is the product of the sarc gene of RSV. Therefore, a protein has been identified as the gene product responsible for the malignant transformation of Rous sarcoma virus. The purification of the protein and elucidation of its function should provide great insights into the mechanism of carcinogenesis by RNA tumor viruses.

The protein responsible for the leukemogenic potential of the Friend strain of spleen focus-forming virus (SFFV), a highly potent leukemogenic virus has been identified for the first time. Last year in our laboratory the structure of this virus was studied. SFFV was recognized as being a recombinant virus just as the Kirsten and Harvey murine sarcoma viruses are recombinant viruses. The genes acquired by the spleen focus-forming virus during the recombination which led to its malignant potential were identified and in the past year the gene product of these newly acquired recombinant genes was identified. In the case of the Rous sarcoma virus, the sarc protein can be shown to be coded for directly by the recombinant sequences defined at the genetic level as the sarc gene. Similarly, the genetic sequences acquired by the spleen focus-forming virus which code for a newly identified protein were also derived by the mechanism of recombination. In no other system in RNA tumor virology has the gene product of the genes acquired by recombination in the rapidly transforming viruses been identified. The protein identified in the spleen focus-forming virus is 6-70,000 daltons in molecular weight and has been identified as a glycoprotein. It is located at the cell surface and a radio-immunoassay has been developed which detects this protein. Studies are in progress to purify this protein and define its biological function. The isolation of this protein provides a biochemical hallmark in leukemogensis research. It allows the identification for the first time of a protein responsible for the conversion of a normal hematopoietic precursor to a leukemic cell. Because it is a glycoprotein, and located at the cell surface, readily apparent models come to mind as to how it may work, and how scientists might design a strategy of either preventing leukemic transformation, or killing the cells once they become transformed. Because of its location at the cell surface,

immunologic approaches to prevention and therapy might be fruitful. The laboratory effort over the next few years will be focused on characterizing this protein and attempting to develop some practical approaches to prevention and therapy, the identification of similar molecules in human leukemia, and the elucidation at a molecular level of how this protein produces its leukemogenic effect.

The second major effort in the Laboratory of Tumor Virus Genetics involves the application of recombinant DNA technology to the genomes of RNA tumor viruses. It is now possible through the work that has been done in DNA recombinant research in the past few years to clone part or all of RNA tumor virus genomes in E. coli. Such experiments can provide quantities of viral genes impossible to obtain by any other method to allow detailed characterization of the structure and therefore functions of critical RNA tumor virus genes. The technology for performing these experiments has been developed now in the Laboratory of Tumor Virus Genetics with the aid of an Expert Cancer Consultantship. The research will be conducted under the NIH guidelines for Safety and will be directed at answering several questions in different model RNA tumor virus systems.

1. The mammary tumor virus of the mouse has been shown in this laboratory to have its expression regulated by glucocorticoid hormones. The sequences in the virus necessary for its responsiveness to glucocorticoids will be identified by recombinant DNA technology and these promoter genes will be sequenced. Since hormonal influences on a variety of human epithelial tumors is striking, knowledge at the genetic level of how steroid hormones regulate and produce their effects should provide new tools with which to approach the question of hormonal therapy and control of human cancer.

2. The sarc and leuk genes of the transforming RNA tumor viruses noted above will be cloned, and the primary structure of these genes will be elucidated. This work should complement the protein work that is ongoing in the laboratory and provide insights into how the virus synthesizes its transforming proteins. The equivalent genes in DNA tumor viruses, such as SV40, code for the T-antigen. The synthesis of T-antigen has turned out to be a complex process based on a comparison between the gene products and the primary structure of the gene. Thus, it will also be important in RNA tumor viruses to look at genes vs gene products since insights into the synthesis of different forms of T-antigen has come only from knowing the sequence of the gene as well as having the protein itself.

3. In the field of RNA tumor virology it has become apparent that as much as 0.1% of the genome of rodents or primates is comprised of retroviral genomes. This astronimically high number stands in stark contrast to the paucity of isolates of helper independent viruses (viruses able to replicate) from either rodents or primates. These results indicate that the majority of genomes of retroviruses in any of their species of origin are replication-defective. Therefore, conventional approaches (e.g., co-cultivation) employed to isolate competent RNA tumor viruses from most species have a statistically calculable low probability of success. However, DNA recombinant technology can bypass the need for this work in the isolation of genes of these defective viruses. DNA probes synthesized from available viruses have in most cases, even in

primates, detected some related genes in species in which no virus can be isolated. These species include man. Using DNA recombinant technology, we will attempt to isolate from high primates including man, related genes of these replication-defective endogenous viruses. If this can be accomplished then the need for isolation of virus by conventional methods will have been bypassed.

4. Recombinant DNA technology will attempt to construct new types of recombinant RNA tumor viruses, inserting into them perhaps, non-retroviral genes in an attempt to use nontransforming retroviruses as vectors for gene therapy in the future.

The Laboratory of Tumor Virus Genetics has been in existence for two and a half to three years. It inherited a group of investigators already in existence at NCI at its inception. Over the past three years it has effectively reprogrammed the available personnel that were given to the laboratory at its inception, redirected their efforts and brought the laboratory to an efficient usage of these personnel and its available resources. The problems that it is investigating have reached an extremely promising stage of research. Major intellectual insights have been gained into the very mechanism of carcinogenesis, and the application of the technology of DNA recombinant research to critical problems in retroviral research has been developed. In addition to the research indicated above, members of the Laboratory of Tumor Virus Genetics have been involved in extramural activities which further indicate the prestige and acceptance of the work that the laboratory has conducted. Two of the members of the Laboratory of Tumor Virus Genetics are members of the editorial board of the Journal of Virology, and one is a member of the editorial board of Virology. The chief of the laboratory is the senior editor for all tumor virus papers in the Journal of Virology, the major publication of the American Society for Microbiology for tumor virus research. The laboratory members have remained active in participating in the Virus Cancer Program of the National Cancer Institute and its various committees. The laboratory has shown an excellent balance between basic molecular work and its application to biologically important questions. It has consistently demonstrated its ability to address itself to the important questions in cancer research and to utilize or develop appropriate technology to attack these difficult problems.

Birthplace of Gene Therapy

SUMMARY REPORT

B. 2. d. LABORATORY OF VIRAL CARCINOGENESIS

October 1, 1977 - September 30, 1978

The Laboratory of Viral Carcinogenesis (1) plans and conducts research on virus-host relationships in virus-induced cancers with emphasis on the detection and characterization of oncogenic viruses and the mode of viral transmission in animals and man; (2) studies the interaction of viral and cellular genes; (3) studies host immune mechanisms related to the control of virus-induced cancers; and (4) conducts investigations on the molecular processes of viral carcinogenesis. Intramural and collaborative research efforts employing interdisciplinary approaches have produced improved understanding concerning the nature of the cellular transformation phenomena. Several significant laboratory results have brought certain types of human cancer within range of more meaningful laboratory and clinical etiological research. Clinically oriented results obtained by this Laboratory will be summarized first, followed by those with clear implications for human cancer and last, those exploring the basic etiological and host mediated mechanisms of malignant transformation.

Clinically Oriented Findings:

Cancer skin test antigens with greatly increased sensitivity were produced for use in human cancer patients. The skin test antigens were prepared from mass cultures of human cancer cells that have been infected with a non-pathogenic virus that buds from the tumor cell membrane. The combination of the virus antigens with the tumor antigens in the membrane markedly increases the strength of the tumor antigens for skin testing purposes. In a recent clinical study Crude Membrane (CM) extracts from three different cultured human melanoma lines that were "virus-augmented" (infected with vesicular stomatitis virus and subsequently inactivated by ultraviolet light) produced positive skin tests in 17 of 20 (85%), 11 of 20 (55%), and 13 of 18 (72%) tests, respectively, performed in 20 melanoma patients. Identical CM extracts that had not been infected with virus gave positive skin tests in 2 of 20 (10%), 4 of 20 (20%), and 2 of 18 (11%) tests, respectively. The three virus-augmented extracts were positive in less than 6% of tests in control subjects with other cancers or normal volunteers. Thus, virus-augmented CM extracts showed markedly greater sensitivity without significant loss of specificity as compared to nonvirus-augmented extracts. Studies in animals support the possibility that the virus-augmented cancer skin test antigens may also be useful in the treatment of melanoma, possibly carcinomas, and as markers for tumor detection and treatment effectiveness.

A successful treatment regimen for inflammatory breast cancer was identified. A cooperative project between the NCI and the Institute Salah Azaiz (ISA), Tunis, Tunisia, initiated a chemotherapy trial on a rapidly progressing form of breast cancer which has only a 20% five year survival rate. Characterized

by edema and inflammatory signs, this form of breast cancer has been proven to be unresponsive to conventional therapy (surgery and radiation). Utilizing combination chemotherapy consisting of 5-fluorouracil, methotrexate and cyclophosphamide, investigators treated 39 patients with this fulminant form of breast cancer and obtained a significant tumor regression in more than 75%. Because Tunisian breast cancer patients are almost evenly divided between those with the inflammatory form of breast cancer and those with the more common form of disease comparable to that seen in most American patients, the investigators were able to develop a study which can evaluate those features which determine the progression of breast cancer in patients from all countries. As a result of this study, which included 581 histologically verified breast cancer patients seen in five years at ISA, in addition to showing the remarkable response to chemotherapy, it was shown that there is a contribution of environmental factors (rapidly progressing breast cancer was observed in rural patients more than urban) and genetic factors (Blood group A was significantly more frequent in inflammatory breast cancer, a finding comparable to familial breast cancer in the United States) in the etiology of rapidly progressing breast cancer in Tunisia. These studies are important to breast cancer patients (and possibly to other cancer patients) in the United States because the availability of a group of patients with highly aggressive disease makes it possible to identify more rapidly those forms of treatment likely to be useful in breast cancer patients from all countries. Furthermore, the identification of factors involved in the development of rapidly progressing breast cancer may help in the prevention of this cancer type in U.S. patients, who currently appear to develop this phase in less than 5% of cases. Since the vast majority of patients on chemotherapy had marked regression or complete disappearance of tumor, a comparison of factors distinguishing responders from non-responders will lead to an improved means of selecting breast cancer patients for specific chemotherapy protocols.

An indication was found through three separate studies that genetic factors may be closely responsible for the immune response to Epstein-Barr virus (EBV). The first study was a coded comparison of 210 clinically normal individuals from multiple-case cancer families and age/sex matched controls. A significantly higher EBV antibody level in the multiple-case cancer families confirmed previous studies demonstrating a link between familial cancer and high EBV titers. The second study, showed that normal individuals and patients with Hodgkin's disease who had the B27 HL-A histocompatibility antigen on their leukocytes, had a significantly depressed immune response to Epstein-Barr virus. The third showed, in collaboration with investigators at the University of Singapore, that high antibody dependent lymphocytotoxicity to EBV and high titers to HL-A antigens in NPC patients correlated with prolonged survival. These studies are important: 1) Epstein-Barr virus has been shown to cause a number of human diseases, including infectious mononucleosis, Guillain-Barre syndrome, abacterial tonsillitis, blood dyscrasia, in addition to being suspected of causing Burkitt's lymphoma and nasopharyngeal carcinoma. This link to the genetic control of EBV provides a possible explanation as to why this one virus results in such a spectrum of illnesses. 2) Because EBV is such a ubiquitous virus, the identification of

a link between immune response to the virus and any illness allows the development of a test for susceptibility to these diseases. Since genetics are known to affect survival in patients with a variety of tumor types, the link between genetics and EBV titers in EBV associated diseases (such as NPC) may lead to improved monitoring of therapy and selection of better treatment regimens.

Studies on nasopharyngeal carcinoma (NPC) in the United States utilizing a newly developed American NPC registry and classification of 207 cases, improved diagnosis and management of NPC patients in the United States. Affecting approximately 2000 Americans each year, NPC has been studied extensively in the Far East and Tunisia but only recently has a determined effort been made in this country. Review of 207 American NPC cases accessioned at the Armed Forces Institute of Pathology indicated that the most common form of NPC in American Caucasians and American blacks is of the undifferentiated form, the type most commonly found to contain Epstein-Barr virus genome in studies in other countries. Performed in collaboration with AFIP pathologists, the study indicated that blacks in the United States have a higher percentage of undifferentiated carcinoma than whites, that a significant number of young Americans (blacks and whites, but particularly blacks) are affected by NPC, and that the newly developed World Health Organization classification system for NPC is readily applicable to US cases. In spite of occasional EBV antibody differences between patients in different countries (including the United States, Tunisia, Hong Kong and France), patients in general were quite similar in their antibody patterns when compared with matched normal and cancer controls from the same countries. Thus the identification of specific etiological factors can more readily be obtained by international comparisons of NPC patients. The applicability of a uniform histological criteria, one which has not previously existed and which had led to great confusion in the American literature (17 histologic classifications have been pared down to three major types), will allow greater comparability between US and foreign studies, thus permitting better identification of similar and different etiologic factors in American and foreign cases. Young adult cases, now being identified in the U.S., can be studied particularly closely since they will provide a better opportunity to identify etiologic factors. These young adult cases are very rare in the high incidence areas, such as China and Hong Kong, where genetic factors are believed to have the highest impact on the incidence of NPC, and thus chemicals, viruses (particularly EBV), and other environmental factors may be identified more readily. The availability of EBV antibody assays associated with the NPC Registry can be used to improve the diagnosis and monitoring of NPC cases in the United States.

Findings Closely Applicable to Human Cancer

Uncontrolled growth of malignant cells is related to the altered activity of certain hormone-like substances with the cell surface. The interaction of specific receptor sites with certain growth factors causes an overstimulation of cell division which results in abnormal multiplication. Genes such as the "src" gene in mouse sarcoma viruses and in cells transformed by them, may

code for a substance(s) related to Epidermal Growth Factor (EGF). Normal mouse epithelial and fibroblastic cells will bind EGF to their unsaturated receptors and initiate cell division. Sarcoma virus transformed mouse cells will not bind EGF because their receptors are saturated or altered. The products made by the transformed cells have been partially purified. This model has been applied to human sarcoma using a similar substance, Multiplication Stimulating Activity (MSA). Normal human fibroblasts will bind MSA while (transformed) human sarcoma cells will not bind MSA. These studies indicate that MSA and other polypeptide growth hormones may be gene products involved in the transformation process. A human fibrosarcoma cell line in culture has been found to produce a family of MSA-related peptides which stimulate cell division in different species. Purification, characterization, and measurement of these substances will be useful in (a) early diagnosis of specific types of human cancer, (b) provide means to improve monitoring for treatment effectiveness and prognosis, and (c) open new avenues for improved understanding of the etiology of cancer.

Three new viruses, recently isolated from primates closely related to man (langur, owl and stumptail monkeys) were biochemically and immunologically characterized. They are endogenous, genetically transmitted viruses in each species; thus the viral information was detectable in the DNA of normal cells. It was further demonstrated that related species have related viral genes in their DNA and that those viral gene sequences have evolved as the species evolved. These sequences are contained in the DNA of both New World and Old World primates. These isolates have brought the total of genetically transmitted viruses of primates to 5 (3 type C and 2 type D). The fact that viruses have been isolated from man's closest relatives and that related DNA sequences have been detected in human DNA, suggests that as technology improves and experience is gained, other primate species, including man, will most likely yield these types of endogenous oncogenic viruses. The existing isolates have made possible the development of biochemical and immunological assays for markers related to viral gene expression in a variety of human normal and malignant tissues. Each new virus isolate allows the development of specific reagents (nucleic acid probes, antigens, enzymes) with which human populations may be examined for the expression of viral genetic material. Two additional new type C viruses were isolated from mice originating from Southeast Asia. The unusual finding concerning these isolates was their demonstrated preferential relationship to primate type C viruses (woolly monkey and gibbon ape) rather that to the classical murine type C oncogenic viruses. The fact that endogenous viruses are genetically transmitted in animal species has served to demonstrate the evolutionary progression of oncogenes in the various species, including primates and man, and how interspecies transmission and recombination of oncogenic viruses may occur. This work is significant because it has provided a sound basis for the understanding of the interaction between environmental and genetic aspects of cancer etiology, susceptibility, and resistance. The number of classes of different viruses carried as mouse genes is extensive. Two families of type C viruses and two families of type B viruses are carried in the genetic material of all species of mice.

Nerve Growth Factor (NGF) interacts with cell types embryologically derived from neural crest, including sympathetic and sensory ganglia and neuroblastoma. Melanomas in culture were shown to have high levels of specific receptors for NGF probably reflecting their ancestral origin. Some melanomas possess abundant receptors while others have variable quantities. Classification of clinically distinguishable classes of melanoma on the basis of NGF receptor availability is being studied. While patients with melanoma do not have significantly different levels of circulating NGF when compared with controls, the marked difference in receptor site concentration and specificity on the malignant cell surface offers a potentially fruitful means for improved diagnosis and treatment. It may become possible to identify human melanoma cells in vivo by methods that detect NGF receptors.

A new human chromosomal locus Bevi has been identified and assigned to human chromosome six. Bevi (for baboon endogenous viral integration) was shown to dominantly control baboon endogenous virus infection and replication in human cells. This was demonstrated by baboon virus infection and subsequent analyses of over 160 human x rodent hybrid clones segregating human chromosomes. Subsequently, baboon virus preinfected human cells were fused to Syrian hamster cells and the resulting hybrids analyzed for continued viral replication. Representative $Bevi^+$ and $Bevi^-$ hybrids were examined for the presence of baboon proviral DNA sequences in hybrid cellular DNA by employing nucleic acid hybridization techniques. These results demonstrated that Bevi is a preferred integration site for baboon endogenous virus in the human genome. Somatic cell hybrids derived from more than 6 independent fusion experiments were infected with three retroviruses other than baboon endogenous virus. In addition, human cells preinfected with these same viruses were fused to rodent cells. All of the resulting hybrids were analyzed for continued viral replication and chromosome composition. Results suggest that genetic loci involved in viral replication can be identified and localized for each of these retroviruses.

Basic Viral Oncology Findings:

Sarcoma virus transformed cells have been shown to release a family of polypeptide growth factors, designated Sarcoma Growth Factors (SGFs), into the supernatant fluids in cell cultures. They stimulate cell division, compete for Epidermal Growth Factor (EGF) receptors on the cell surface, induce normal fibroblasts to grow in soft agar, and to express some of the properties of transformed cells. While the murine sarcoma virus produces a permanent genetic change in the cells it transforms, the SGFs produced only reversible phenotypic changes that depend on the continued presence of the factors. These cells can be cycled back and forth between the untransformed and transformed phenotype, without apparent genetic change, by either growing them in the absence or presence of the SGFs. The in vitro response of cells exposed to the factors is similar to that produced by murine sarcoma virus transformation. It appears that the SGFs act as effectors of fibroblastic transformation. The new SGFs are not produced by untransformed cells or by DNA transformed cells. The endogenous production of polypeptide growth factors by cells that are able to respond to their own products may represent

a general mechanism for cell transformation. The SGFs appear to be the first direct effectors of cell transformation, taking cell morphology and anchorage independent growth in agar as indices of the transformed phenotype. These growth factors represent the first instance in which a peptide produced by sarcoma virus transformed mammalian cells causes normal cells to assume the in vitro properties of transformed cells. This work also provides further support for the hypothesis that murine sarcoma virus transformation involves the production of polypeptide growth hormones. In addition, further support is given to the general model of cell transformation in which viruses provide genes coding for an exogenous protein, or in which viruses act by allowing the expression of cellular genes which are normally repressed.

Sarcoma virus producing mammalian cells were compared to nonproductively infected systems. In each of four different mammalian species the number of proviral copies of MSV "sarcoma specific" sequences was increased between two- and tenfold. There was a corresponding increase in "common" sequences of MSV in each system. In contrast, the same mammalian cells infected with only helper viruses demonstrated one to three copies per haploid genome. Feline sarcoma virus (FeSV) specific nucleotide sequences as well as those sequences shared with feline leukemia virus (FeLV) were examined, cDNA's were purified which represented each subset. Two different FeSV sarcoma specific sets of sequences were found. Sequences in "common" with FeLV were covalently linked to these "sarcoma specific" nucleotide sequences. FeSV "sarcoma specific" sequences were found in normal cat DNA and were conserved in evolution. Nucleotide sequences of FeSV in common with FeLV were found only in species closely related to the domestic cat and diverged rapidly. The isolation of viral nucleotide sequences which induce and maintain transformation serves as a useful tool to determine the mode of interaction of viral sequences with cellular macromolecules and to serve as an adjunct for identifying its putative protein product. The variety of mammalian sarcoma viruses with different "sarc" sequences poses interesting new questions relative to how many such sequences exist and whether there are common target sites in cells.

Revertants of MSV transformed cat cells were examined for the presence of MSV "sarcoma specific" and "common" sequences with purified cDNA representing defined regions of the genomes. Neither MSV "sarcoma specific" or "common" proviral DNA was found. Endogenous feline xenotropic virus proviral sequences were unchanged in quality or number in revertants. Endogenous feline leukemia-like sequences were similarly unchanged in cat revertant cell DNA.

This laboratory was the first to describe the natural occurrence of "recombinant" murine leukemia viruses (MuLV). These are composed of genetic information derived from two or more different groups of MuLVs. These viruses, which have a very wide host range, appear to be the real disease inducing agents in the mouse. Such recombinant viruses were clone-purified and their oncogenicity in mice was demonstrated. The same recombinant viruses were isolated from the induced leukemia; no other MuLVs were present. Purified leukemia-derived virus was again able to induce new disease in

recipient hosts, fulfilling the criteria for causality. Under natural conditions the recombinant virus was generally not detectable whereas related virus(es) were present. It became clear that the recombinant virus was present but that it was "masked" in the sense that it borrowed the coat of related viruses against which the mouse's natural defense mechanisms were not adequate. This explained why such recombinant viruses were not isolated earlier. The site (the gene) in which recombination took place was localized. The site was the major envelope glycoprotein which confers host range, interference, and neutralization properties. This glycoprotein was analyzed by a number of techniques including digestion with proteolytic enzymes followed by "fingerprint" maps which could characterize it. These fingerprints of the recombinant viruses were unique and the presumptive parents were identified. The changes in the region of this gene appear to confer leukemogenic potential to the virus but the precise mechanisms are not as yet known. The isolated recombinant viruses could arise by changes within a gene or by substitution of whole genes. The specific identification and isolation of genes involved in cellular transformation should be helpful in determining how these genes bring about the neoplastic state.

Certain chemically transformed mouse cells as well as those transformed by DNA-containing tumor viruses (e.g. SV-40) produce common DNA-binding regulatory proteins; normal cells do not contain these specific binding proteins. Two independently isolated chemically transformed cell lines, one treated with DMBA, the other with benzopyrene, appear to make closely related, if not identical, new DNA-binding proteins. These will preferentially bind to mouse cell DNA and not to human cell DNA. The experiments suggest that different chemical carcinogens may effect common growth regulatory systems within the cell and may act like DNA-containing viruses in inducing new DNA-binding proteins. Antiserum prepared to these purified proteins will allow testing for their presence in "spontaneous" as well as chemically induced tumors.

Non-murine cells have been productively infected with several high oncogenic mouse mammary tumor viruses (MMTVs). Newly synthesized MMTV can be detected as early as four days post infection and provides the basis for a rapid in vitro assay for MMTV. In addition, this system should provide a means to study the potential co-carcinogenic effects of MMTV and physical or chemical carcinogens.

MMTV proviral sequences were detected in the cellular DNA of mammary tumors and livers or RIII and C3H mice by molecular hybridization using radioactively labeled MMTV 60-70S RNA. By means of DNA:DNA reassociation kinetics, the DNA of the mammary tumor cells of these two mouse strains were found to contain more MMTV proviral sequences than the DNA of liver cells of these same tumor bearing mice. ^{125}I labeled MMTV (C3H) 60-70S RNA was annealed to a vast excess of DNA from C3H livers of pooled organs, and single stranded RNA was eluted from hydroxylapatite and recovered. This "recycled RNA" did not hybridize to the DNA of the apparently normal organs tested from normal C3H mice, but hybridized extensively to the DNA from spontaneous C3H mammary tumors. Similar experiments were conducted with the RIII mouse

strain. We have, therefore, isolated the sequences of the RNA genomes of the MMTVs from C3H and RIII mice that are transmitted by some mechanism other than via the germ line. These sequences are present as proviruses in the DNAs of mammary tumors of high incidence strains, but are not detected in the DNAs of mammary tumors of the moderate and low incidence strains. These sequences are not detected in the DNA of apparently normal livers of GR mice. These experiments provide direct biochemical evidence that there are more than one MMTV. We have also demonstrated that a small difference exists between the nucleic acid sequences of the highly oncogenic MMTVs.

The polypeptides of three primate type D retroviruses - Mason Pfizer virus (MPV), langur virus (LV), and squirrel monkey retrovirus (SMRV) - were compared using both sodium dodecyl sulfate polyacrylamide slab and tube gel electrophoresis. The polypeptides of MPLV and LV could not be distinguished by their electrophoretic mobilities. The major polypeptide of both MPV and LV was approximately 27,000 d. The other polypeptides found in both MPV and LV had estimated molecular weights of 70,000, 47,000, 36,000, 30,000, 12,000, and 10,000. The polypeptide pattern of SMRV was easily distinguished from those of MPV and LV. The major polypeptide of SMRV was approximately 36,000 d. Other SMRV polypeptides had molecular weights of approximately 75,000, 47,000, 32,000, 22,000, 20,000, 17,000, and 12,000. RIAs were established for the major internal proteins of these type-D retroviruses. Neither of the homologous RIAs for p27 of MPV or p27 of LV could be used to distinguish MPV from LV. The reactivity of SMRV in the LV p27 RIA was greater than in the MPV p27 RIA. Sera to LV was also used to precipitate MPV p27, thereby establishing an interspecies radioimmunoassay for Old World type D retroviruses. Radioimmunoassays have also been established for two low molecular weight proteins of type D retroviruses. The RIAs for the MPV p10-12 and the LV p10-12 could easily distinguish these two viruses.

The feline gene Bvr-1 (BALB virus restriction) which reversibly restricts constitutive production of B-tropic BALB endogenous mouse virus in cat x mouse hybrid cells was characterized. Bvr-1 also restricts induction of BALB virus-2 (xenotropic), but not BALB virus-1 (N-tropic) by iododeoxyuridine. These observations suggest that the targets of Bvr-1 in the N-tropic virus are distinct from those of the B- and xenotropic viruses. Bvr-1 is late acting at the budding stages of MuLV production. Infectivity studies with residual virus produced in Bvr-1 restricted cells raise the possibility that Bvr-1 interferes with the post transcriptional processing of ecotropic murine endogenous virus. A possible human locus homologous to Bvr-1 is suggested of B-tropic MuLV in mouse x human somatic cell hybrids.

Cocultivation of primate tissues with mink cells nonproductively transformed by Kirsten sarcoma virus led to the isolation of an endogenous mink type C virus designated MiLV. MiLV-related sequences were readily detected in the normal cellular DNA of certain other carnivores (e.g. ferrets, ermines and skunks) while less related sequences were identified in evolutionarily unrelated rodent species (i.e. mice and rats). Immunological data showed that the p30 protein of MiLV is closely related to that of feline leukemia

virus (FeLV) which is presumed to have an ancestral origin in rodents. The lack of further homology between MiLV and FeLV suggests that both viruses originated in rodents but were independently transmitted to ancestors of present-day carnivores.

Several examples of new types of murine endogenous RNA tumor viruses were isolated from mouse species from Southeast Asia. The two new isolates were unrelated to the classical murine type B and type C oncogenic viruses. Newborn mice were readily infected by the isolates as were mouse cells in culture. This virus is closely related in many of its properties to the mouse mammary tumor virus. This isolation of viruses from wild populations of different rodent species shows that the breast cancer class of viruses is not restricted to laboratory mice. Isolations of this type are significant because they serve to certify to the ever improving techniques for virus detection in, and isolation from, mammalian cells and to a better understanding of the role of various types of oncogenic viruses in the function, development, and evolution of cancer in various species, including man.

Chemical carcinogen induction of certain tumors results in concomitant expression of antigens of endogenous type C viruses. The expression of viral specific antigens is linked with rapid cell reproduction. Experiments with rats, performed in collaboration with scientists at the M.D. Anderson Hospital in Houston, Texas, showed that primary tumors expressed only low levels of viral antigens but secondary transplants and subsequent passages progressively increased in their yield of viral antigens as they increased their ability to grow and to spread. This is significant because certain definable viral marker proteins may be useful as indicators of secondary tumor growth and of progressively de-differentiated tumor cells.

In addition to their intramural research activities, many of the senior investigators within this Laboratory spent a substantial portion of their time in support of the Virus Cancer Program. These investigators served as Project Officers, Assistant Project Officers, members of coordinating committees and in other supporting capacities. The activities of the Viral Cancer Program and those of this Laboratory are aimed at the common goal of the determination of the viral etiology of human cancer. While the efforts of this Laboratory's scientists on behalf of the VCP have significantly contributed to its progress to date, the broad scientific perspective developed by these investigators in their VCP activities contributed greatly to the direction of the program of this Laboratory. This interaction will continue to underscore the pursuit of the goal of understanding the suspected interaction between tumor viruses and human malignancy. Such a goal will be approached only through the combined efforts of experts in widely differing disciplines within this laboratory and those of collaborating laboratories.

SUMMARY REPORT

C. 1. a. COLLABORATIVE RESEARCH BRANCH

October 1, 1977 - September 30, 1978

The Collaborative Research Branch of the Viral Oncology Program, Division of Cancer Cause and Prevention, was established to participate in the planning, development and scientific administration of a program of collaborative research conducted within the Virus Cancer Program on viruses as etiologic agents of cancer in man and animals and on the control of tumor viruses and/or their induced diseases.

The Breast Cancer Virus Studies Section manages contracts conducting research on viruses as a cause of mammary carcinoma in animals and the elucidation of their role in breast cancer in humans with emphasis on the study of virus-like activity demonstrable in human case materials and on the reproduction, characterization, composition and cellular transformations associated with viruses known or suspected to cause mammary gland carcinomas. The Clinical Studies Section manages contracts conducting research (a) to provide evidence for viral associations with neoplasia in human cohorts, (b) to investigate host factors in humans and animals which may modify or otherwise influence susceptibility to infection or the development of neoplasia following infection, (c) to develop methods which may be applicable in prognosis or diagnosis of malignancies, and (d) to investigate methods which may be effective for the control of infection or development of neoplasia in animals and humans. The Cocarcinogenesis Studies Section manages contracts conducting research on the interaction of viruses with chemical, physical and biological environmental agents as cofactors causing neoplastic transformation of mammalian cells, emphasizing (a) development and standardization of cell-virus systems for study; (b) identification of the interactions which result in transformation; (c) determination of the mechanisms involved; and (d) definition of methods for prevention or control of induction of transformation. The DNA Virus Studies Section manages contracts conducting research on viruses with a DNA core which are known or suspected to be involved in the induction of malignant transformation of animal and human cells and supports studies on (a) elucidation of the role of viruses of this category in the induction of neoplastic disease, (b) their characterization and biological activity, (c) intracellular relationships established with cellular components, (d) virus genome expression and intracellular control, (e) mechanisms of reproduction and induction of neoplasia, and (f) inhibition of replication and cell transformation. The RNA Virus Studies Section manages contracts conducting research on viruses with a ribonucleic acid core which are known or suspected to induce malignant transformation of animal and human cells with primary emphasis upon agents which exhibit characteristics of viruses known to induce leukemia, lymphoma, sarcoma and related diseases in laboratory animals, and supports studies in (a) the detection in case material of activities or components characteristic of viruses of this nature, (b) virus isolation and characterization, (c) virus replicative process, (d) intracellular relationship between virion and cellular components, (e) virus genome expression and intracellular control, (f) mechanisms of virus-induced cell transformation to malginancy, and (g) inhibition of replication and cell transformation.

SUMMARY REPORT

C. 1. b. (1) BREAST CANCER VIRUS STUDIES SECTION

October 1, 1977 through September 30, 1978

The Breast Cancer Virus Studies Section is concerned with research to determine whether viruses are etiologically involved in human mammary gland malignancies. The approach has been to study several animal model systems for evidence of viral oncogenesis. Such animal studies are necessary for developing technology and approaches to search for a putative human breast cancer virus.

Mouse Mammary Tumor System: At the present time, breast cancer is known to be caused by a virus in only one animal species, the mouse. Several projects in this section are investigating the genetic transmission of mouse mammary tumor viruses (MuMTV) and trying to identify the loci responsible for its expression. Thus far, at least three genes appear to influence MuMTV in mouse mammary tumorigenesis. A single dominant gene (designated Mtv-1) controls the release of a low oncogenic variant of MuMTV in C3Hf mice while a second gene, Mtv-2, determines expression of a highly oncogenic virus strain in GR mice. It is believed that the third, a repressor gene, inhibits the release of a mammary tumor provirus integrated into the genome of most, if not all, mouse strains.

Results of a three year breeding project designed to test the hypothesis concerning the control of endogenous MuMTV support the presence of the three genes.

As the loci responsible for genetic variants of endogenous MuMTVs are identified, congenic mouse strains are produced, which have specific genetic deletions, so that linkage studies for gene mapping can be performed. Recently, a congenic GR/MTV-2-strain has been established, which lacks the MTv-2 locus, does not express MuMTV in milk and does not develop early mammary tumors. These mice will be a useful control in studying the oncogenic action of Mtv-2.

In other studies, it was found that the Mtv-1 gene of the foster-nursed strain is not allelic with Mtv-2 and is located on the first linkage group between c and the Gp-1 locus. Three-point crossings are under way to map precise locations of all MuMTV genes.

Another approach to studying the regulation of MuMTV gene expression in mammary tumorigenesis utilizes specific cDNA probes to quantitate levels of MuMTV RNA and casein mRNA sequences in RNA extracts isolated from mouse mammary tissues. Preliminary data indicate that MuMTV and casein gene expression are not regulated coordinately during normal or mammary neoplastic development and that hormonal regulation of both gene products appears to change during preneoplastic to neoplastic transformation. Further studies utilizing an *in vitro* organ culture system to define specific hormonal factors influencing virus expression are underway.

Although the successful infection of non-murine cultured cells with MuMTV a few years ago represented a technological breakthrough for mouse mammary tumor virology, virus infection and replication are still inefficient in these cells. Recently, a contract was initiated to establish more efficient in vitro epithelial culture systems for studying the infectivity of MuMTV. Thus far, a C57Bl normal mammary gland cell line and two human breast cancer lines have been successfully infected. Significant progress on the mouse mammary tumor virus structure, assembly and maturation has been made. It has been shown that the glycoproteins of MuMTV, gp52 and gp36, are on the envelope surface, the non-glycosylated polypeptides, p28 and p14, are core proteins and that p10 is membrane associated. It was further hypothesized that during maturation it is the p10 (as a lipophilic external coat of an intracellular A-particle) which links the MuMTV nucleocapsid precursor to the envelope.

The complexities of viral carcinogenesis and host-tumor interactions are being investigated. Evidence has been presented that natural cell-mediated immunity against mouse mammary tumor virus is independent of thymus-derived (T) lymphocytes. In other studies, it has been reported that immune complexes of MuMTV antigen-antibodies are responsible for nullifying the cellular response of T-lymphocytes in virus positive tumor-bearing mice. Immunologic reactivity could be restored by repeated washing of T-cells with mild tryspin.

A new study implemented this year will determine whether environmental factors can induce mammary cancers in low tumor incidence mice by activating, either partially or fully, endogenous MuMTV sequences. In vivo and in vitro studies are being conducted on the effect of chemicals and x-irradiation on MuMTV-RNA expression in BALB/c and BALB/cf C3H mice. A second project will utilize pituitary implants to generate hormone-induced early mammary tumors in virgin BALB/c females. Expression of MuMTV structural antigens will be monitored by nucleic acid hybridization techniques.

Investigations on the efficacy of nucleic acid-free viral vaccines against the onset of mammary tumorigenesis in mice are being pursued. It has been reported that vaccination of DBAf mice with purified MuMTV, gp52 or p28, had no protective effect against challenge with virus-laden tumor cells, but injection of low doses of membrane fraction preparations elicited considerable protection. Present studies are underway to determine whether chemical modification of MuMTV antigens will provide better results. In studies designed to increase the immunogenicity of isolated mouse mammary tumor viral antigens, immune complexes of gp52-anti-gp52 which are coupled to inert, phagocytosable carrier particles are being used.

Rhesus Monkey-MPMV System: When it was discovered in 1969, the Mason Pfizer monkey virus (MPMV) was cited as the first primate oncornatype virus to be isolated from a spontaneous breast carcinoma of a rhesus monkey. Since then, several reports in the literature have suggested that RNA copies and antigens cross-reactive with MPMV are found in mammary tumor tissues from human patients. Because of its importance as a possible

model for human breast neoplasia, MPMV-infected rhesus monkeys have been extensively investigated. Infectious MPMV has been reisolated from the milk and saliva of p27 anti-genemic monkeys as neonates 4 to 7 years previously. No virus was recovered in the plasma, urine or feces of antigen-positive animals and uninoculated control animals have remained virus-free. Interestingly, not all antigenemic monkeys had infectious virus in the milk. Only those animals which received x-irradiation in the breast during puberty showed infectious particles. Preliminary data seem to suggest the possibility of x-irradiation involvement in the activation of latent MPMV infection in certain antigen-positive monkeys.

In the other studies, the transforming potential of MPMV in primate and human cells is being investigated. The specific objective is to isolate conditional and non-conditional transformation-defective mutants of MPMV for use in analyzing primary gene products, which may be responsible for cellular transformation.

New Mammary Tumor Systems: Although breast cancer occurs in many different mammals, the mouse is the only species in which a viral etiology for breast cancer has been definitely established. Since important differences exist between the experimentally induced mammary tumors in inbred laboratory mice and the naturally occurring spontaneous breast tumors in humans, it was felt that there was a need for more research in other animal models, which more closely mimic the outbred populations of man and which have cancers more analogous to those found in humans. Malignant epithelial breast neoplasms in dogs are quite similar to human breast disease in morphology, clinical behavior and incidence. A concerted effort was made to utilize tissue techniques for the isolation of a virus from mammary tumors that arise spontaneously in dogs. Canine mammary tumor cultures were propagated under the influence of a variety of hormone and chemical-virus inducers and co-cultivated with cells from a number of homologous and heterologous species. This approach led to the isolation of a new retrovirus from a cocultivated dog and mink culture. Recent characterization studies demonstrated the virus to be of mink origin. The search for viral information in other canine mammary tumors is being conducted.

Feline breast cancer accounts for approximately 17% of all neoplasms in female cats and, like human breast neoplasms, it is a disease of the middle age. An attempt has been made to isolate a virus from feline mammary tumors and to determine whether viruses can cause breast cancer in cats. Seventy feline breast tumors have been examined for virological activity by electron microscopy, tissue culture, immunological and biochemical techniques. Virus-like particles, morphologically similar to intracisternal A particles and immunologically unrelated to any mammalian oncornaviruses, have been observed in approximately 60% of the tumors examined and in three established mammary lines. Cell cultures containing the particles have a 20 hour doubling time, a chromosome modal number of 37 and are tumorigenic when injected into Swiss/Nude mice. Thus far, kittens inoculated with extracts from tumor cells have not developed breast neoplasia. The animals are being kept under observation.

Human Mammary Tumor System: The search for viral association with human breast malignancies has continued. Indirect evidence has accumulated that human mammary tumors contain immunologic reactivity which appears to be antigenically related to the mouse mammary tumor virus. One study demonstrated that lymphocytes from postoperative breast cancer patients react quite specifically to preparations of RIII-MuMTV and gp55 in the leukocyte migration inhibition assay. Positive reactivity was not found with preparations of RIII-gp68, gp34 and p28. Similar protein fractions from other known oncornaviruses (such as MPMV, RLV) were also negative. An extension of these studies has revealed that tumor eluates from selected patients contain a 50,000 MW protein which immunologically cross-reacts with RIII-gp55 and a p50 isolated from MCF-7, a human breast tumor cell line. The degree of relatedness of all of the reactive fractions is being characterized by standard immunochemical and biochemical techniques.

Although the 734B particle, an agent with retrovirus-like properties, was first isolated from MCF-7 culture supernatants in 1974, subsequent replication has been sporadic and unstable in that cell line. A major focus this year has been to define controls which regulate 734B synthesis in MCF-7 cells and to manipulate these controls so that sufficient quantities of 734B can be replicated for its complete characterization. Studies conducted thus far have shown that the expression of an antigen cross-reactive with the gp52 of MuMTV seems to be correlated with the release of 734B. A clone of MCF-7 cells with a high level of antigen expression has been isolated and attempts are now being made to identify those factors which regulate its expression.

Studies to compare the ultrastructural features of normal, benign, atypical and malignant human mammary epithelial cells suggests that certain ultrastructural makers are more prevalent in malignant and atypical cells and these markers may be useful in identifying women with early disease. The significance and validity of these observations are being evaluated.

CONTRACT REPORTS

BREAST CANCER VIRUS STUDIES SECTION

Dr. Clarice Gaylord

ALABAMA, UNIVERSITY OF (NO1-CP7-1013)

Title: In Vitro Transforming Potential of MPMV

Contractor's Project Director: Dr. Eric Hunter

Project Officer (NCI): Dr. Robert Bassin

Objectives: To determine the role of Mason-Pfizer monkey virus (MPMV) in the transformation of primate and human cells.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: MPMV, isolated from a female rhesus monkey, is an important primate retrovirus since it is infectious for both primate and human cells. Since preliminary data indicate that MPMV can cause malignant transformation of rhesus cells in culture, this putative carcinoma virus is an ideal candidate for studying those viral genes that may be involved in cellular transformation and carcinoma formation. An understanding of the mechanisms of viral-induced primate and human cell transformation will clearly be useful in the development of improved treatment and possibly prevention and control of breast cancer in humans.

Proposed Course: Conditional transformation-defective mutants of MPMV will be isolated in efforts to identify those viral genes responsible for cell transformation.

Date Contract Initiated: September 20, 1977

BAYLOR COLLEGE OF MEDICINE (NO1-CP4-3385)

Title: Regulation of Gene Expression in Mouse Mammary Cancer

Contractor's Project Director: Dr. Susan H. Socher

Project Officer (NCI): Dr. Steven Tronick

Objectives: Examine the regulation of gene expression in cells using murine mammary tumor virus (MuMTV) RNA and casein mRNA as specific markers of gene function in mouse mammary tissue. Analyze the expression of these individual genes in normal mammary tissues, preneoplastic alveolar nodules and mammary tumors in both MuMTV-positive and MuMTV-

negative lines of mice. Quantitate levels of MuMTV and casein RNA sequences in RNA extracts isolated from these tissues by means of hybridization to cDNA probes selective for MuMTV and casein mRNA. Specific emphasis is being placed on the mechanisms by which hormones modulate transcription of these genes.

Major Findings: During normal mammary gland development the level of casein mRNA increased during pregnancy and lactation and fell following weaning. Mammary glands from the BALB/cfC3H line had higher levels of MuMTV RNA than the BALB/c line. Although the percent of MuMTV RNA increased during pregnancy and lactation in BALB/cfC3H mice, it did not decline after weaning. Casein mRNA and MuMTV RNA are not regulated coordinately during mammary gland development.

Hyperplastic alveolar nodules and mammary tumors of both the C- and D-series, induced in BALB/c mice by treatment with hormones and/or carcinogens, contained appreciable amounts of MuMTV RNA. Low levels of casein mRNA have been detected in all nodules and tumors assayed.

The hormonal regulation of MuMTV RNA and casein mRNA expression have been studied in BALB/c mice bearing D-2 nodules and D-2 tumors. Prolactin alone and in combination with hydrocortisone increased the level of casein mRNA in D-2 nodules and not in D-2 tumors. Hydrocortisone alone increased the level of MuMTV RNA in D-2 tumors but did not increase the percent of MuMTV RNA in D-2 nodules. Thus, the hormonal regulation of MuMTV and casein expression appears to change during the preneoplastic to neoplastic transformation.

The characterization of an organ culture system has been used in defining the specific hormonal factors regulating MuMTV expression. Of particular interest is the in vitro observation that hydrocortisone increased the level of MuMTV RNA in pregnant tissue from BALB/cC3H mice and in D-2 tumors from BALB/c mice.

Significance to Biomedical Research and the Program of the Institute: Considerable evidence suggests that control of the inherited mouse mammary tumor virus genome occurs at the level of DNA transcription to RNA. These studies are designed to obtain an increased understanding of the regulatory mechanisms of the effects of hormones in differentiation and on the regulation of viral and nonviral genes involved in carcinogenesis.

Proposed Course: Future efforts will focus on examining the effects of peptides and steroid hormones on MuMTV and casein gene expression during normal and neoplastic mammary tissue development. In addition, mammary gland explants maintained in organ culture will be utilized to dissect the multiple hormone interactions that modulate gene expression.

Date Contract Initiated: June 26, 1974

BAYLOR COLLEGE OF MEDICINE (NO1-CP8-1006)

Title: Effects of Environmental Factors on Endogenous MuMTV Expression in Low Mammary Strains of Mice

Contractor's Project Director: Dr. Janet S. Butel

Project Officer (NCI): Dr. Howard Young

Objectives: To induce MuMTV expression and mammary tumor development in mice having a low incidence of spontaneous mammary neoplasms by the use of chemical carcinogens and x-irradiation.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: It is well known that hormones, chemicals and ionizing irradiation may enhance the development of cancer. These same factors also seem to influence expression of oncogenic RNA tumor viruses and host cell transformation. Knowledge of the extrinsic factors which can affect viral gene activation may provide information leading to the control of neoplasia.

Proposed Course: Chemical carcinogens (NMU and DMBA) and x-irradiation will be employed singly and in combination to activate or derepress endogenous MuMTV expression. Manipulations with these environmental factors will be performed *in vivo* at different stages of mammary gland development and on cells in culture. Observed changes in virus expression or cellular properties will be correlated with the incidence of mammary tumor development and transplantability *in vivo*.

Date Contract Initiated: January 13, 1978

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CP6-1013; successor to NO1-CP3-3253)

Title: *In Vitro* Cultivation of Human and Mouse Mammary Tumor Virus

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. Robert Bassin

Objectives: To develop techniques, reagents and concepts in the mouse mammary tumor system which might be applicable to the human breast cancer problem.

Major Findings: A human "breast cancer specific antigen" was examined for MuMTV related reactivity. The human antigen and antisera directed against it did not cross react with MuMTV by the radioimmunoassay, immunodiffusion

or immune precipitation tests. It also suggested that the human protein was not related to MuMTV.

Application of the "M" protein salt-detergent extraction procedure resulted in the purification of p10 in the "M" protein pellet and established the hydrophobic and lipophilic nature of p10. It was hypothesized that p10 may be closely associated with the viral envelope; thus, a model of MuMTV maturation with p10 as the molecule which links the nucleocapsid to the envelope was proposed.

An immunoperoxidase method for the detection and localization of MuMTV antigens in paraffin embedded tissue sections at the light microscope level was developed. The procedure demonstrated that MuMTV antigens are not uniformly distributed in virus-infected tissues.

Determination of the basal levels of viral mRNA and vDNA in MuMTV free BALB/c strain and MuMTV-carrying BALB/cfC3H mice has been completed. Levels of viral mRNA and vDNA in the D1 and D2 hyperplastic outgrowths transplanted into BALB/c and BALB/cfC3H strains were determined and the results have been analyzed. Various MuMTV-negative and MuMTV-positive hyperplastic outgrowth lines have been initiated.

Significance to Biomedical Research and the Program of the Institute: The acquisition of knowledge and technology on the in vitro cultivation of mammary gland tissue and the production and structure of MuMTV is essential to an intensive program of investigation on virally-induced breast cancer in experimental systems and for application in virological studies on human breast cancer.

Proposed Course: A major effort this year will be the virological investigation of the preneoplastic state in the mouse mammary gland. Studies will be continued on MuMTV structure and the isolation and characterization of the ribonucleoprotein.

Date Contract Initiated: February 1, 1972

ENERGY, DEPARTMENT OF - LAWRENCE BERKELEY LABORATORY (Y01-CP7-0510; successor to N01-CP5-3502)

Title: Studies on Human Mammary Tumor Virology

Contractor's Project Director: Dr. Adeline J. Hackett

Project Officer (NCI): Dr. John Dahlberg

Objectives: To develop and characterize human mammary epithelial cell lines representing the various stages in malignant progression. To identify morphological and biochemical markers which may correlate with each state of malignant breast disease.

Major Findings: One breast tumor metastatic to bone and one metastatic to skin are in early stages of cell line development. Short-term cultures of normal, benign and malignant mammary cells have been achieved from frozen pools of ductal and alveolar "organoids" isolated from breast tissue.

Cell substrates frozen at early passage, carefully characterized and identified morphologically, ultrastructurally and biochemically, will be utilized as the source material for development of established cell lines.

A quantitative analysis of expression of ultrastructural markers for malignancy has been developed. This procedure distinguishes normal, pre-malignant and malignant human breast cells.

Studies on cell-to-cell interactions show promise to develop into an approach for understanding the role of myoepithelial cells in the normal functional activity of the breast and in benign and malignant disease processes.

Preliminary study of lactate dehydrogenase isoenzyme patterns show correlation of altered isoenzyme (L5/4) ratios in malignant mammary epithelial cells, both in culture and from breast fluids.

Significance to Biomedical Research and the Program of the Institute: The development of cell lines provides the substrates required to compare and standardize various assays which measure the biological properties of cells. Cells in culture are uniformly growing and usually less differentiated than primary specimens, thus providing an unobscured view of the differences in stages of malignant progression. Human mammary cell lines, representing the various stages of malignancy as well as the various types of breast cancers, will facilitate future studies on the genetics and immunology of mammary cancer, as well as help to clarify the nature of the variability in clinical expression of breast disease.

Proposed Course: Continue to use human mammary epithelial cell lines to search for new parameters which might distinguish between cells at various stages of malignant progression. The search for viruses which may be associated with cancer of the breast will begin by comparing surgical specimens with cells growing in culture.

Date Contract Initiated: September 1, 1974

HAHNEMANN MEDICAL COLLEGE (NO1-CP8-1007)

Title: Studies on Mammary Tumor Viruses

Contractor's Project Directors: Dr. Dan H. Moore
Dr. Akhil B. Vaidya

Project Officer (NCI): Dr. Ernest Plata

Objectives: To infect established mouse and human mammary epithelial cell lines in vitro with MuMTV and to determine if the susceptible cells have specific receptors for MuMTV or its major surface glycoproteins.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: In determining the possible involvement of an agent similar to mouse mammary tumor in human breast malignancies, it is necessary first to expand our knowledge about the mouse model system. The development of new in vitro systems to study the infectious process of MuMTV at the cellular level and the regulation of the expression of MuMTV proviral genes will aid in understanding tumorigenesis by this virus and possibly other related agents.

Proposed Course: This study proposes to perform a series of molecular biological and immunological studies to determine the viral and cellular requirements for infection of murine and human epithelial cells by MuMTV.

Date Contract Initiated: February 1, 1978

INSTITUTE FOR MEDICAL RESEARCH (NO1-CP8-1003)

Title: Study of Common Antigens in MuMTV, Human Milk and Breast Tumors

Contractor's Project Director: Dr. Arnold S. Dion

Project Officer (NCI): Dr. Dante Marciani

Objectives: To compare antigens found in human breast tumors and milk with structural polypeptides of MuMTV by various biochemical and immunological techniques.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: It is imperative to determine whether the reported presence of human MuMTV-related antigens can be corroborated. If similarities do exist and a viral association with human breast cancer can be established, this finding will be important in the diagnosis and possible immunoprophylaxis of human breast neoplasia.

Proposed Course: Major structural proteins of MuMTV, p50(s) from breast tumor eluates and MuMTV-related antigens from human milk will be isolated

in quantity and purified. Biochemical characterizations, such as molecular weight determinations and assessment of homogeneity by X-terminal analyses will be performed and the immunological relatedness will be determined.

Date Contract Initiated: December 1, 1977

MASON RESEARCH INSTITUTE (NO1-CP6-1052; successor to NO1-CP3-3358)

Title: Studies on Mammary Tumors in Rhesus Monkeys Infected with Mason-Pfizer Monkey Virus

Contractor's Project Director: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To determine whether MPMV is oncogenic in monkeys.

Major Findings: Demonstration of active infection, as indicated by MPMV p27 antigenemia, in the majority of MPMV-inoculated subhuman primates being held and monitored for mammary tumor induction has been completed. The incidence of infection in the various primate species was as follows: of the rhesus monkeys inoculated with infectious MPMV as neonates, 96% (27/28) were antigenemic, and of those inoculated as juveniles or adults, 64% (14/22) were antigenemic; in other primate species antigenemia was detected in 7 of 8 cynomolgus inoculated as neonates, in 4 of 4 cynomolgus and 5 of 5 bonnets inoculated as juveniles and in neither of 2 owl monkeys also inoculated as juveniles. In comparison, all 19 uninoculated rhesus controls were negative..

Infectious MPMV has been reisolated from intact exfoliated mammary cells present in the milks of p27 antigenemic rhesus monkeys inoculated 4 to 7 years previously. Free filterable infectious virus was not found. This potential for horizontal transmission of MPMV via the milk is supported by a case history documenting an antigenemic offspring resulting from the mating of a nonantigenemic father and a mother which was inoculated with MPMV postpartum (during lactation) and became antigenemic while suckling the offspring.

Not all antigenemic monkeys have exfoliated mammary cells that produce infectious virus. A review of clinical histories revealed a relationship of infectious milks with those animals whose breasts were X-irradiated during puberty, suggesting X-irradiation and mammotrophic hormones as cofactors in the activation of latent MPMV in breast tissue of antigenemic animals.

Preliminary screening of saliva, urine and feces of antigenemic animals revealed MPMV p27 antigen in saliva and urine and infectious virus in a cell-free filtrate of saliva, the latter indicating an additional mode for horizontal transmission.

An antibody to MPMV in the sera of antigenemic and nonantigenemic animals inoculated with infectious MPMV was demonstrated using fixed immunofluorescence with Clone 4 cells. Seven out of 10 sera (70%) from rhesus inoculated as neonates, and 1 out of 18 sera (5%) from rhesus inoculated as adults were antigen positive antibody negative. On the other hand, antigen negative antibody positive sera were found in 10 out of 18 rhesus (56%) inoculated as adults and in only 1 out of 10 rhesus (10%) inoculated as neonates. Of particular interest, 18% of sera tested were antigen positive antibody positive indicating both virus antigen expression and an immune response in the same animal.

Significance to Biomedical Research and the Program of the Institute: MPMV was recovered from a primate mammary cancer, it possesses characteristics common to known oncogenic viruses, and cross-reactions have been observed between the viral antigens and antigens present in human breast cancer specimens. Investigation for possible oncogenic properties in a primate host is valuable as a potential model for human breast cancer.

Proposed Course: Continuation of the studies described and collection of data necessary for decisions concerning continued long-term holding and observation of these animals.

Date Contract Initiated: June 9, 1970.

MIAMI, UNIVERSITY OF (NOI-CP5-3532)

Title: Immunologic Studies on Animal Breast Carcinoma

Contractor's Project Director: Dr. Michael M. Sigel
Dr. Diana Lopez

Project Officer (NCI): Dr. Meera Paranjpe

Objectives: (1) To determine, define and integrate the relevant factors in host defense against mammary tumors; (2) to analyze cellular immunity in relation to clinical state; and (3) to compare lymphocyte responses to MuMTV-associated, MuMTV-induced and non-MuMTV tumor-specific antigens in mice with breast cancer.

Major Findings: In a series of experiments it was shown that the magnitude of the response of the B cells to MuMTV-associated antigen(s) was increased in tumor-bearing mice. This differential responsiveness was observed in several instances but was by no means uniform. The failure of T-cells to respond to MuMTV antigens was viewed either as an inherent attribute of T-cells or as a deficit caused by depletion of auxiliary cells such as macrophages. In order to test this latter point several experiments were performed in which T-cells and B-cells separated in nylon wool were confronted with MuMTV antigen(s) in the presence and absence of peritoneal exudate cells (PEC) induced intraperitoneally by oyster glycogen injections

in normal syngeneic BALB/c mice. The results showed that T-cells remained unresponsive even in the presence of such cells.

In previous reports the occurrence of T-cells with surface markers has been usually associated with B-cells. Such cells were not found in normal spleens but became prominent after the establishment of tumors following transplantation of minced tumor fragments. Splenocytes from tumor bearing animals were separated on nylon columns and T-cells were studied in a triple marker assay for the simultaneous presence of Thy 1.2 antigen, CR and FcR by means of immunofluorescence and rosetting techniques. There were no cells within the nylon nonadherent population which possessed the CR and FcR markers simultaneously. Thus, it appears that the CR and FcR markers occur on separate T-cells and are not co-expressed on T-cells as they are on B-cells. After transplantation, tumor development and cytotoxic activities were measured over a period of 4 to 5 weeks. Tumor extirpation or sham surgery (distal to the tumor site) were performed at 4, 7, 14 and 21 days following tumor implantation and 7 days after each operation splenocytes were tested in three types of cytotoxic reactions: PHA-induced cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC) and specifically-induced nonspecifically-expressed cytotoxicity (SINEC). The results confirmed the previous findings that all three reactions are elevated in tumor-bearing animals. The increase in ADCC activity could be reversed by removal of tumor. This effect paralleled the reversal in the increase of Thy 1+FcR+ cells.

In further experiments normal- and tumor-bearing mice were immunized i.p. with SRBC at different time intervals following tumor transplantation. Six days after immunization part of each group of animals was killed and their spleens assayed for IgM and IgG plaque-forming cells by the Cunningham procedure. Another part of each group was injected at the same time with SRBC in the foot pad in order to evoke a skin reaction which was measured at 3 hours for immediate and 24 hours for delayed hypersensitivity.

The results indicate that there was significant increase in the number of IgM and IgG plaque-forming cells in the spleens of tumor-bearing animals after 18 days of tumor implantation. In contrast the delayed hypersensitivity was significantly decreased compared to normal animals. Splenocytes from tumor-bearing mice were exposed in culture to corresponding tumor extracts and the supernatants of these cultures were tested for cytotoxic activity against chicken red blood cells (CRBC) by the chromium release method. Two kinds of assays were performed. In one, supernatants alone were added to the target cells while in the other the supernatants were first added to fresh normal splenocytes which were then added to target cells. The results demonstrated that a low level of lymphotoxic-like activity could be detected in the supernatants by the direct test.

Significance to Biomedical Research and the Program of the Institute:
The murine mammary tumor viruses are etiologically related to breast cancer in mice and there is some evidence that similar viruses may be present in humans. Because of the laboratory and clinical similarities between breast cancer in mice and in women, the present work is designed to obtain

a clearer understanding of the immunological reactivity of the host to an array of MuMTV and tumor-related antigens. The approach is to identify various antigens and immunologic as well as nonimmunologic factors governing the relationship and interactions of host, virus and tumor. This project will provide information relevant to both etiology and control of breast cancer in humans.

Proposed Course: This study will continue to assess the immunologic status and response in mice with different virological and immunological backgrounds and to correlate these responses to various clinical stages of mammary gland neoplasia.

Date Contract Initiated: March 15, 1974

MICHIGAN CANCER FOUNDATION (NO1-CP3-3347)

Title: Studies of High Risk Breast Cancer Families

Contractor's Project Director: Dr. Marvin A. Rich

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To characterize virus particles (734B) produced by the human breast tumor cell line, MCF-7, and to identify and characterize human populations at high risk for breast cancer, seeking to provide early detection and control of breast cancer.

Major Findings: MCF-7 cultures were shown to be a mixture of cell types in dynamic equilibrium and only one small subpopulation, comprising less than 10% of the MCF-7 cells, expressed an antigen cross-reactive with MuMTV. The cell population which expressed the antigen is morphologically distinguishable from the bulk of the population which does not. The cells which express antigen are called L-cells; the nonexpressor population, S-cells. Progesterone augments antigen expression in L-cells, but does not induce antigen synthesis in S-cells or in mixed L/S populations.

L- and S-cell clones were generated by the limiting dilution method in microwells so that clonal purity could be directly established microscopically. Clonal populations have been expanded to mass culture for characterization. In the course of these studies, the cross-reactive antigen in MCF-7 cells has been shown to be related to the major external glycoprotein of MuMTV, the gp52.

During the current contract period, it was established that the L-variant of MCF-7 proliferates as a malignant outgrowth in athymic mice, that the malignant outgrowth is hormone-dependent and that continuous exposure to abnormally high levels of estrogen is the primary tumor-promoting influence.

Considerable progress has been made in making long, representative probes from MuMTV and MPMV. cDNA molecules with a molecular weight of 2.2×10^6 daltons from both viruses have been synthesized and the results confirm and extend earlier reports on the homology of nucleic acid sequences of MuMTV and MPMV with human breast tumor RNA's. The present experiments have also shown homology between MCF-7 RNA and cDNA's from both MuMTV and MPMV.

The leucocyte migration inhibition reactivity of patients with documented breast cancer to identical extracts of MCF-7 and D-562 (control antigen) was found to differ markedly. The percentage (58%) of patients responsive to MCF-7 antigens was similar to that reported by others. The specificity of the reactivity was also supported by finding that only 1 of 13 healthy individuals responded.

New techniques developed in the current contract year have resulted in a substantial improvement in the growth of the normal cells in culture. Epithelial cells in such cultures divided extensively and generated large colonies. One ml of postweaning fluid yielded about 3×10^5 proliferating normal mammary epithelial cells. These epithelial cells in primary cultures can be successfully transferred into 2nd and 3rd passages using techniques of mechanical and proteolytic dissociation and feeder layer cultures.

Over 65 cultures of normal human mammary epithelial cells were tested for the expression of MuMTV-related antigens and were found to be negative. Thus, there appears to be a clear distinction between the expression of MuMTV-related antigens in normal versus malignant cells. The relationship among these various activities and their ultimate influence on breast carcinogenesis remains to be elucidated.

Significance to Biomedical Research and the Program of the Institute: These studies concern the isolation and characterization of a possible human breast cancer virus. Reagents prepared against any viral related components present in MCF-7 cells would be a valuable diagnostic tool.

Proposed Course: Future efforts include the selection of a high producer variant from the heterogenous MCF-7 cell population for the purpose of generating sufficient quantities of 734B virus for further characterization. Additional lines of human breast cancer cells will be developed for maintaining high levels of virion replication in culture.

Date Contract Initiated: June 20, 1971

MICHIGAN CANCER FOUNDATION (NO1-CP8-1001)

Title: Effects of Environmental Factors on Expression of Endogenous MuMTV in Low Mammary Strains of Mice

Contractor's Project Director: Dr. Charles M. McGrath

Project Officer (NCI): Dr. Robert Callahan

Objectives: To determine whether exogenous mammotropic hormones can change MuMTV expression in mammary cells and whether the change correlates with tumor onset and incidence in low mammary strains of mice.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: It may be possible to regulate human breast tumor incidence by controlling the hormonal environment of the host. This study is designed to elucidate the role of viral-hormonal interactions in the generation of mammary tumors and attempts will be made to manipulate both factors in vivo.

Proposed Course: Proposed studies are to quantitate mammary tumor onset in virgin BALB/c females primed and unprimed with hormones from pituitary implants and to determine whether MuMTV RNA is induced in mammary cells during malignant transformation and whether the induction correlates with tumor incidence.

Date Contract Initiated: February 1, 1978

NETHERLANDS CANCER INSTITUTE (NO1-CP3-3368)

Title: Immunogenetic Studies on Breast Cancer and Leukemia

Contractor's Project Directors: Dr. L. M. Boot
Dr. J. Hilgers

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To study the immunogenetics of mouse mammary tumors and MuMTV transmission, as well as that of leukemia and murine genes for transmission, MuMTV replication, histocompatibility, and immune response.

Major Findings: The GR/Mtv-2 strain does not develop pregnancy-dependent mammary tumors and the Mtv-2 locus, equivalent to 6 MuMTV DNA copies, has been shown to be responsible for such early mammary tumors in the GR strain. The congenic strain has 3-4 copies of MuMTV DNA left per haploid genome and also expresses low levels of MuMTV RNA and antigens.

A number of somatic cell hybrids between GRSL cells and E36 Chinese hamster cells, segregating mouse chromosomes, retain MuMTV DNA copies. Assignment of such copies to specific chromosomes is now under way.

Histocompatibility genes of the D-end of the H-2 locus control resistance to mammary tumorigenesis by exogenous C3H-MuMTV. So far no H-2 effects have been found in systems where mammary tumorigenesis is the result of endogenous MuMTV.

Mammary tumors induced by hormonal manipulations, such as transplantation of hypophyses, in the BALB/c, C57BL and 020 strains, do not contain increased MuMTV DNA and MuMTV RNA contents indicating that MuMTV may not be involved in the genesis of these tumors.

Two strains of mice of European origin (STS and TSI) have been shown to resist mammary tumorigenesis by the most optimal hormonal induction methods, making it possible to study the genetics of resistance to hormone-induced tumors.

A survey of inbred strains for the occurrence of antibodies to MTV in normal mice by the RIP assay showed that MuMTV expression among low mammary cancer strains is much more widespread than previously thought, but expression usually does not occur before the end of the reproductive period. The presence of the milkfactor leads to an increased occurrence of natural antibodies to MuMTV.

The MLr antigen is present on the MuMTV gp/3 env precursor of MTV as well as the MuMTV gp52 part of the molecule; the gag precursor of MTV and its products are not expressed on the cell surface of GRSL cells. The MLr antigen is the only "modulable" antigen on these cells and the modulation process is very specific in the sense that other cell surface antigens (H-2K, Thyl.2, TL, etc.) do not show a change in expression after modulation of the viral antigen.

Significance to Biomedical Research and the Program of the Institute: Study of genetic factors controlling host susceptibility and capacity for internal control of oncogenic virus expression and infectivity is important to the program of this Institute. The results obtained thus far begin to delineate a variety of factors, in addition to the presence of an oncogenic virus, which contribute to or modify the pathogenesis of mammary cancer or leukemia. The findings described contribute to the investigations of possible etiological agents of human cancer as well as to greater understanding of the fundamental biology of cancer.

Proposed Course: The contractor proposes to continue studies concerning host genes involved in MuMTV and MuLV expression in relation to mammary tumorigenesis and leukemogenesis and studies concerning viral antigen expression at the cell surface level and the immune response directed towards these antigens.

Date Contract Initiated: June 28, 1972

NEW YORK MEDICAL COLLEGE (NO1-CP3-3398)

Title: Immunologic Measurements as a Guide to the Behavior and Viral Etiology of Breast Cancer

Contractor's Project Director: Dr. Maurice M. Black

Project Officer (NCI): Dr. Robert Callahan

Objectives: (1) To identify tumor- and virus-specific antigens in human breast cancer tissues; (2) to correlate measurements of patient immunologic reactivity with clinicopathologic characteristics of the tumor tissue and the host; (3) to search for evidence of the role of a mammary tumor virus in human mammary carcinogenesis by immunologic and biochemical procedures using MuMTV as a probe (in collaborative studies).

Major Findings: Extended studies of microscopically demonstrable lymphoreticuloendothelial (L-RE) responses to breast cancer tissue suggested that immunologically mediated tumor retardation played a role in the biological behavior of human breast cancer. It further appeared that the prognostically favorable immunological responses were cell-mediated rather than antibody-mediated. It was also shown that structural evidence of tumor immunogenicity and prognostically favorable cell-mediated immunity were most regularly demonstrable in Stage 0 in situ carcinoma cases.

The possibility that some breast cancer tissues contain a component which is similar to RIII-gp55 was also suggested by the finding that extracts of L-RE-positive breast cancer tissues commonly contain a 50,000 MW protein whose charge density is similar to that of gp55. The cellular reactivity of breast cancer patients' leukocytes to a variety of purified protein fractions of RIII-MuMTV and the gp50 fraction of A-MuMTV was examined. It was found that breast cancer patients' leukocytes were selectively responsive to RIII-gp55 as compared with RIII-gp68, gp34 and p28. Moreover, cellular reactivity to RIII-gp55 was poorly correlated with reactivity to A-gp50.

Studies of leukocyte migration inhibition reactivity of more than 200 breast cancer patients tested (1 to 6 times) simultaneously against RIII-gp55 and autologous breast cancer tissue suggest that breast cancer tissues have a gp55-like antigen. Of particular clinical significance was the finding that cross-reactive cell-mediated immunity to gp55 and autologous breast cancer tissues was preferentially found in prognostically favorable cases in general and in in situ cases in particular.

Significance to Biomedical Research and the Program of the Institute: Since the findings of this effort strongly suggest that the in vivo and in vitro cellular hypersensitivity responses of breast cancer patients provide biologically significant indices of maximal tumor-host interactions in the initial stages of disease, the techniques used by the contractor may be useful tools in the possible early detection, diagnosis and effective treatment of breast cancer in women.

Proposed Course: To broaden the data base regarding postoperative monitoring of individual breast cancer patients for cellular reactivity to autologous breast cancer tissues and viral related antigens; to study the antigenic properties of breast lesions in relation to such risk factors as age, parity, family history, oral contraceptives and replacement estrogen therapy, and to isolate and better characterize the cell-mediated immunity determinants found in human breast cancer tissue which appear to be cross-reactive to MuMTV antigens.

Date Contract Initiated: June 26, 1972

PFIZER, INC. (NO1-CP6-1054)

Title: Studies of Viral Involvement in Canine Mammary Carcinoma

Contractor's Project Director: Dr. Mumtaz Ahmed

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To obtain evidence concerning the association of viruses with mammary neoplasias in dogs of various breeds.

Major Findings: A retrovirus (MiRV) antigenically distinct from known type C, B and D viruses was isolated from a culture following cocultivation of dog mammary tumor cells with a mink lung cell line. Karyotypic analysis of the cocultivated culture demonstrated a normal mink (Mustela vision) chromosome composition with the exception of a missing #9 chromosome and a low frequency of extensive chromosomal breaks and coiling. MiRV bands at a buoyant density of 1.16 - 1.17 gm/ml, contains 60-70S RNA and a transcriptase which prefers Mn^{++}over Mg^{++} for its activity. This enzyme utilizes poly (rA) more efficiently than poly (dA) and is also able to synthesize DNA copies from the endogenous RNA. Morphologically, it is a typical type C virus with a central dense nucleoid (50-60 nm) surrounded by an electron lucent envelope (100-110 nm). Hybridization studies designed to determine the origin of MiRV demonstrated that proviral sequences complementary to this virus are present in normal mink DNA indicating that MiRV is endogenous to mink. Filtered MiRV readily infects mink and dog cells indicating the amphoteric nature for its growth.

MiRV isolation was the result of a systemic periodic monitoring of more than 175 cultures (primaries, cocultivated and activated) that were generated from 28 dogs. With the one exception mentioned above, all cultures failed to show any significant and persistent viral polymerase activity. Virus-like particles were observed in mammary tissues of 8 dogs, but actively multiplying dog virus was not isolated from dog cells in spite of hormonal or chemical stimulation and cocultivation with cells of homologous or heterologous species. Immunofluorescence tests demonstrated evidence of type C virus interspecies determinants in 15 cultures from 12 dogs, but type B or type C related antigens were not detected in any of the 90 different cultures that were screened.

Significance to Biomedical Research and the Program of the Institute: At present, the study of virus-induced mammary carcinoma is restricted to the mouse. Breast cancer is one of the most prevalent types of human cancer and recent studies suggest that a virus may be associated with the disease in humans. Breast cancer is also among the most common neoplasms of dogs and resembles the human cancer in morphology, clinical behavior and incidence. Study of this malignancy in canines permits application of

present knowledge of this carcinoma beyond the restriction of the murine system and increases prospects for increased understanding of viral roles in the human disease.

Proposed Course: To continue to examine mammary tumors, pleural effusions and milk secretions from several breeds of dogs for viral related information. Dog tissues established in culture will continue to be cocultivated with cell lines of homologous and heterologous species and treated with chemicals in attempts to activate a putative viral genome.

Date Contract Initiated: June 25, 1976

RADIOBIOLOGICAL INSTITUTE (NO1-CP4-3328)

Title: The Study of the Release of RNA Tumor Viruses and Its Genetic Control

Contractor's Project Director: Dr. Peter Bentvelzen

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To carry out studies *in vivo* and *in vitro* to elucidate the role of hereditary factors controlling the expression of genetically transmitted mouse mammary tumor viruses of mice. The importance of such genes in spontaneous mammary carcinogenesis will be evaluated in relationship to environmental agents such as chemical carcinogens and ionizing radiation.

Major Findings: Glucocorticoids have been reported to induce early mammary tumors, which contain a potent mammary tumor virus, MuMTV, in low cancer strain mice. Tumor development was thought to be due to suppression of immunological control of such a virus. When treated with glucocorticoids, kidney cultures of C3Hf mice release a type B oncornavirus which induces tumors at an early age. This result suggests that C3Hf mice carry genetic information for a potent MuMTV which is usually strongly repressed but is readily activated by glycocorticoids. This activation need not be due to immunosuppression.

The MuMTV polypeptide gp36 has now been purified. With antisera directed against the purified polypeptides, it was established by competitive radioimmunoassay that the antigen does not show any cross reactivity, as was observed earlier with less sensitive immunoassays.

The antigen p12 is present on the surface of MuMTV-producing cells; no cytotoxic reaction was observed with anti-p12. This negative result is not due to anti-complement activity of the sera. The indirect cytotoxicity test also failed to induce lysis of the cells. The only target for cytotoxic reaction seems to be gp52. This seems to be considerably better expressed on GRSL18 cells passaged *in vivo* than cells cultured *in vitro* in the presence of dexamethasone.

A microfluorometric enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of MuMTV antigens and heterologous antibodies. This method proves to be considerably more sensitive than the competitive radioimmunoassay or Sepharose bead immunofluorescence assay.

Immunization with purified gp52 or the core protein 28 does not induce transplantation immunity to the L1210 leukemia, which produces MuMTV. With gp52, a moderate dose of antigen even induced acceleration of tumor growth. This proved to be associated with the appearance of blocking factors, as determined with the leukocyte adherence inhibition assay. Vaccination with solubilized virus particles also had no effect, but injection of a low dose of a membrane fragment preparation had a considerable protective effect. Vaccination with purified proteins for prophylaxis of primary tumors seems to be fruitless. Studies are underway to determine whether chemical modification of the antigens may provide better results.

Significance to Biomedical Research and the Program of the Institute: The expression of MuMTV in nonmammary gland tissue and the effect of co-carcinogens on the expression of MuMTV is important to understanding the pathogenesis of virally-induced breast cancer and to prospective prevention or control of this disease.

Proposed Course: To continue the activities described at the current level.

Date Contract Initiated: April 26, 1974

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP6-1053)

Title: Studies to Determine a Viral Involvement in Feline Mammary Carcinoma

Contractor's Project Director: Dr. Nurul H. Sarkar

Project Officer (NCI): Dr. John Dahlberg

Objective: To employ techniques of virus isolation, cell culture, immunology and biochemistry to elucidate a possible viral etiology in feline mammary carcinoma.

Major Findings: Seventy feline mammary tumors have been examined to date for virological activity. Electron microscopic (EM), immunologic, biochemical and tissue culture techniques have been used for this purpose. Virus-like particles, morphologically similar to intracisternal (IA) particles, have been observed in 25 of the 43 (60%) tumors examined by EM. Three epithelial cell cultures derived from feline mammary tumors have been established and characterized. These cell cultures contain virus-like particles, have a doubling time of approximately 20 hours, a chromosome modal number of 37 and are tumorigenic when injected into Swiss/Nude mice. Reverse transcriptase assays of the culture fluid from these cells have revealed a low level of reverse transcriptase activity which can be stimulated by dexamethasone. No specific cross-reactivity between the cells of one of these cultures (and several feline mammary

tumors) and antisera prepared against the feline leukemia or RD-114 virus, MuMTV, MPMV and the gp70 component of the murine IA particle has been detected in any immunological test. Preliminary co-cultivation experiments using two of the epithelial cell cultures and several indicator cell lines did not result in the production ('rescue') of an oncornavirus from these cells. In these experiments the cells were co-cultured for only 2 months. However, many more co-cultivation experiments are being planned for at least 8-12 months.

Significance to Biomedical Research and the Program of the Institute: Currently, the study of virus induction of mammary carcinoma has been confined to mouse systems. Evidence suggests a virus may be associated with human breast cancer. Studies to extend knowledge of viruses in relation to this disease in animal species other than the mouse may yield greater insight into factors which bear upon the human disease.

Proposed Course: Continue to cultivate the feline epithelial mammary tumor cells that have been established in culture (i.e., the K-12, K-4 and K-44 cultures) and establish other normal epithelial and tumor cell cultures. Attempts will be made to isolate virus from these cell cultures using the techniques of chemical activation and co-cultivation.

Date Contract Initiated: June 27, 1976

TEL-AVIV UNIVERSITY (NO1-CP7-1011)

Title: Immunization of Mice with Purified MuMTV Polypeptides

Contractor's Project Director: Dr. Asher Frensdorff

Project Officer (NCI): Dr. Sandra Ruscetti

Objectives: To study the cellular and humoral responses of mice to MuMTV polypeptides and to immunize mice against spontaneous mammary neoplasia with purified MuMTV structural proteins.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: The procedure of preparing vaccines with structurally intact viruses can be dangerous since potentially oncogenic materials can be transmitted as immunogens. This project will explore whether beneficial effects can be achieved from a safe vaccine which does not contain infective nucleic acids.

Proposed Course: Selected female mice will be immunized with an MuMTV nucleic acid-free immunogen complex and observed for occurrence of spontaneous tumors.

The humoral and cell-mediated immunity to viral antigens will be monitored in all immunized and control animals.

Date Contract Initiated: September 9, 1977

SUMMARY REPORT

C. 1. b. (2) CLINICAL STUDIES SECTION

October 1, 1977 through September 30, 1978

The Clinical Studies Section is concerned with viral research related to etiology, diagnosis/prognosis, and prevention/control of human neoplasia. The epidemiology of certain human malignancies are probed through the combined use of laboratory assays and natural history studies, so as to aid in possible identification of factors of resistance and susceptibility to cancer and the identification of environmental and genetic factors which may be involved in the cause of neoplasia. Animal model systems are investigated as diagnostic, preventive and therapeutic potentials for application to human disease.

Sero-epidemiology and natural history studies. The role of Epstein-Barr virus (EBV) as a major factor in the etiology of Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) can be viewed as more than a probability, based on the identification of five new cases of BL in the West Nile district. The total BL cases since the onset of the study stands at fourteen. EBV serology correlated with the cases since pre-BL sera containing EBV VCA antibody were elevated coincident with the tumor manifestation. Such correlation was not found in three cases. These also lacked EBV DNA in the biopsy, suggesting that non-EBV-associated BL's are also found in high risk BL populations. In this population, 5-15% of the children possessed high EBV-antibody titers and were considered to be at a thirty-fold higher risk of developing BL.

Previously, HLA A2B-Sin 2 was reported to be associated with nasopharyngeal carcinoma (NPC) in Cantonese Chinese. Further studies have now identified another HLA genotype (A2BW17) in Chinese and NPC cases in Hong Kong/Singapore, which appears to correlate with short term survivals. Similarly HLA Ag-B-17 was frequently found in Malay NPC patients. No particular HLA association was found in Tunisian NPC cases from an intermediate risk population; however, the frequency of HLA-A2 was high.

The identification of HLA genotypes associated with particular cancers was continued in order to determine the usefulness of HLA typing to identify populations at risk. Weak HLA associations were found in Hodgkins disease (HD) and cancers of the cervix and breast, whereas an HLA type and susceptibility to lymphocytic leukemia and prostatic cancer were more frequent. Associations of other genetic markers such as the D locus, which is manifested on B cells, were identified.

Possible cofactors in NPC and BL, such as consumption of salt fish by the Cantonese Chinese and the prevalence of malaria in endemic BL regions led to investigation of feeding salt fish, rich in nitrosamine, to rats, which developed tumors. Also, the incidence of BL is being closely followed where malaria eradication has been implemented. The poor cellular immunity to EBV in certain normal family members of multiple or single cancer cases, especially in multiple lymphoma indicate that it

may be a useful marker to follow in such individuals. The role of cell-mediated immunity (CMI) in the assessment of resistance and susceptibility was further emphasized with infectious mononucleosis and HD.

Among the animal models studied and considered relevant to the human situation, the existence of vertically-transmitted leukemia viruses in an outbred feral mouse population continues to be investigated. The LC strain of wild mice, which has been geographically isolated for many generations do have a higher incidence of leukemia than wild mice from other locations. Two type C viruses reported from these animals varied in pathogenic manifestations and when transmitted to NIH Swiss mice, both viruses induced lymphoma but only ecotropic virus caused paralysis.

Investigations of the feline leukemia system, another naturally occurring model, suggest that horizontal spread of feline leukemia in household cats was limited to cats; humans in contact with infected animals did not show any immunological evidence of infection. Furthermore, a strong age-related susceptibility to feline leukemia virus (FeLV) and feline sarcoma virus (FeSV) was demonstrated in cats.

A transforming EBV was rescued from nonproducer Raji cells by superinfection with nontransforming EBV. Baboon herpesvirus transformed marmoset and baboon lymphocytes. These lymphocytes failed to exhibit nuclear antigen; however, the virus induced lymphoproliferation in marmosets and resulted in fatal disease. A more oncogenic strain of Herpesvirus saimiri (HVS) was found to consistently induce tumors in the common marmoset. Development of glioma in marmosets with SSV was found to be age-dependent.

Diagnosis and Prognosis. The concept that viruses play a role in oncogenesis is strengthened by the application of viral markers as an aid to diagnosis/prognosis. Two different efforts have concentrated on the application of EBV assays to neoplasia associated with this virus. The IgA antibody to EBV VCA detected in peripheral blood and saliva of NPC patients was helpful in the diagnosis of occult tumors of the head and neck region. The levels of this antibody and other EBV antibodies (EBV VCA and EBV EA) in NPC patients dropped during successful therapy and became elevated before the recurrence of NPC tumor. Specificity of IgA antibody was strengthened by the fact that it was not detected in normal subjects and was present only in low levels in sera from lymphomas and other head and neck carcinomas. Similarly, high levels of antibody-dependent lymphocyte cytotoxicity (ADLC) in NPC cases also correlated to longer survival and favorable prognosis. Blastogenic response to EBV-associated antigens or cells from NPC biopsies in CMI tests were considered useful in prognosis of NPC.

EBNA positive cells were found only in undifferentiated carcinomas of the nasopharynx or the adjacent nasal fossa but not in carcinomas at other sites. Similarly, EBV-related serology correlated well with tests in the BL biopsies. These findings thus provide an added tool for diagnosis of both NPC and BL. The elevation of EBV antibody to EA(R) was poor prognosis in African BL's.

Anti-FLV gp71 serum was found to be useful in differentiating AML and CGL since it specifically reacted with CGL cells. Since the antibody raised to MTV gp52 reacted on breast cancer specimens (obtained from mice and humans), the further assessment of gp52 as a potential diagnostic marker in human breast cancer could be encouraging for future investigations.

Prevention and Control. Prevention and control of viral-induced spontaneous tumors has been attempted by active and passive immunization. Such studies have employed purified viral proteins, antiviral agents, specific antiserum raised to viral products, or the use of viral vaccines free of nucleic acid fragments.

Human interferon is being used in preventing intercurrent viral infection (BK, CMV, etc.) in immunosuppressed kidney transplant patients due to the fact that risk of development of reticulum cell sarcoma or other tumors is greater in these individuals. A nucleic acid-free glycoprotein (gp126) from herpes simplex virus type 2-infected cells in combination with alum adjuvant can provide effective protection in mice against HSV-2-induced paralysis and death. Further testing of this vaccine for immunization of the human population is under investigation. Feline leukemia virus gp75, free of nucleic acid, was administered with alum or adjuvant in pathogen-free cats. In spite of its being a good immunogen, cellular immunity was detected only in half of the vaccinated animals. FOCMA, an antigen associated with cat leukemia/sarcoma, was isolated in culture medium from FL-74 cells and was partially purified. Since the purified antigen was immunogenic, it was thought that it may be a suitable source of antigen for investigations to prevent spontaneous tumors in cats. A vaccine made from tumor cells protected kittens against malignant tumor after oncogenic challenge.

Active immunization with inactivated R-MuLV and passive immunization with goat anti-GLV IgG prevented the expression of endogenous oncornaviruses in x-ray-induced leukemia in mice and also resulted in reduction in tumor incidences. Immunization with gp71 did not protect the AKR mice against spontaneous thymomas or irradiated C57BL/6 thymomas, but it activated an endogenous virus, suggesting that this is a hazardous, active viral glycoprotein immunogen. On the other hand, passive immunization with anti-GLV gp71 caused suppression of endogenous virus and lengthened time of survival. Use of anti-FLV gp71 or disrupted FLV was found to be a successful prophylaxis against FLV infection in kittens.

Leukemic mice treated with interferon had reduced luekemic organ weights, longer life span and reduction in MuLV titers, and such effect was dose related. Cytoreductive therapy combined with interferon was successful in the treatment of AKR leukemia in mice. These investigations further showed that neuraminidase-treated leukemic cells, possessing completely different H-2 genetic loci, were also effective in prolonging the survival

of leukemic AKR mice. Based on this model, the remission in AML patients receiving combined immuno-chemotherapy is already 2 1/2 times greater than that accomplished by therapy alone. The immunodiagnostic tests in vitro and in vivo also support a normal immunocompetence in the combined therapy group.

In evaluating antiviral agents against HVS tumors as a possible model for EBV therapy, phosphonoacetic acid (PAA), a herpesvirus DNA inhibitor, did not provide effective protection against HVS tumors, even though circulating PAA was found in the blood of monkeys and rabbits used for this study. In combination with other antiviral agents and/or immunotherapy, PAA is being evaluated.

In chemically induced tumors, MuLV-associated antigens were demonstrated. Passive immunization of such animals with fetal antigens resulted in significant delay and reduction in MCA-induced tumors. The relationship of embryonic antigens to virus suggested that immunization with syngeneic fetal tissue produced resistance to syngeneic tumor cell transplantation. No natural protection to such transplantation occurred in multiparous animals as compared to virgins. Immunization with syngeneic fetal cells in mice also produced cytotoxic anti-tumor antibody. Such antibody could be specifically absorbed by fetal cell antigen but not by allogeneic murine tumor cells.

Dr. Dharam V. Ablashi

ALABAMA, UNIVERSITY OF (NO1-CP4-3394)

Title: Immunologic Studies on the Relationship of Embryonic Antigen to Virus-Induced Tumor Antigens

Contractor's Project Director: Dr. Eddie W. Lamon

Project Officer (NCI): Dr. Paul Peebles

Ojbectives: Investigation of the cellular immune responses of mice to embryonic and virally-induced antigens of tumor cells by analyses in vitro to determine the magnitude of the response to each class of antigen, the nature of the effector cells, and the mechanisms involved in specific cytolytic responses.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: The determination of the association between virus transformation and the expression of fetal antigens was undertaken to obtain better understanding of the cross-reactivity between viral antigens and tumor-associated antigens which might occur in humans. Understanding of mechanism and characterization of these antigens may lead to more specific assays relevant to tumor etiology and diagnosis. In addition, an understanding of the cellular and humoral responses of the host to these antigens will contribute information which may be important to understanding the mechanisms of viral oncogenesis and to the feasibility of using tumor antigens in immunological control experiments against virus-induced tumors.

Proposed Course: This contract was terminated March 31, 1978.

Date Contract Initiated: June 28, 1974

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP4-3211)

Title: Studies of Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer

Contractor's Project Director: Dr. Paul I. Terasaki

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To detect cellular and humoral immunity in cancer patients and to determine the specificity of these reactions; (2) to understand the

cellular components of the immune response to cancer and its interaction with antibodies in terms of resistance to susceptibility to cancer; (3) to study the immune response of cancer patients and normals to different viruses and virus-induced antigens; (4) to examine the relationship of genetic markers (HLA and others) for linkage to the incidence of cancer in families; and (5) to characterize B lymphocyte alloantigens and to determine their expression in cell hybrids and changes in cells following virus infection or transformation.

Major Findings: Further clarification of the identification of B lymphocyte antigens and more improved methods for testing of these antigens were investigated. Population frequences of B lymphocyte specificities were determined for Caucasians, Negroes, and Japanese. Studies were also performed to show that some antisera reactive against B lymphocytes are markedly more reactive when tested at lower temperature (5°C). Contaminating non-specific cold cytotoxin can be removed from B lymphocyte antisera by adsorption without removing specific antibodies. These cold cytotoxins were found in significantly high levels in patients with several diseases including Burkitt's lymphoma, Hodgkin's disease and other cancers.

A third independent HLA frequency among 526 cancer patients in seven categories, with 629 controls, confirmed that no strong association exists between the HLA-A and -B locus antigens and the types of cancer under study.

Further collaborative studies of possible correlation of EBV serology and HLA frequencies were continued in cancer patients, cancer relatives, and normal subjects. The results indicate that there may be deviations in geometric mean titers associated with B27.

Auto-immune granulocytic antisera were tested from normal persons, chronic myeloid leukemia (CML) patients and CML cell lines. The results suggest that a single antigen determinant called "B"-analogous to the I system on red blood cells may be present on granulocytes.

Effector lymphocytes isolated from nearly all individuals do possess the capacity to kill cultured target cells specifically, an activity now known as natural cell-mediated cytotoxicity. Furthermore, the specificity of this reaction is determined by IgG antibodies already attached to effector cells through FC receptors. These effector cells react with most target cells and give the appearance of a lack of specificity, since natural antibodies are specific for a variety of antigens on most target cells. The methodology for measuring specificities of these reactions have been worked out. Activity to a given antigen needs to be differentiated from all other reactions. These methods are now being applied to the antibody dependent cell-mediated studies of virus-infected cells by use of miniaturized whole blood assay, specifically developed for this purpose.

Initial observations were negative in establishing an association of FeSV infected cells with natural cytotoxic activity from most individuals. Studies involving EBV and other herpesvirus-infected cells initiated recently are in progress. New lymphoblastoid cell lines (46) were HLA typed as A, -B, -C and DRW antigens.

Significance to Biomedical Research and the Program of the Institute: The identification of disease susceptibility genes is of great importance to studies on the etiology of cancer. In family studies, linking genetic type and immunologic response to tumor- and virus-associated antigens may help to determine which humans are more likely to develop specific tumors as well as which abnormal immune responses to certain viral antigens are closely linked to the tumors. These investigators have been involved in studies indicating that individuals with certain HLA types have a diminished response to certain viral antigens. Studies in progress may help to determine whether certain groups of people have an appropriate response to EBV or other suspected tumor viruses. B-cell typing studies are likely to provide useful techniques for characterizing lymphoid cell lines, studying antigenic expression and modulation following virus infection or transformation, and in the diagnosis, prognosis and treatment of various forms of leukemia.

Proposed Course: Studies on familial cancer will continue and, in addition to HLA typing of family members, lymphocyte defined antigens will be measured. An attempt will be made to obtain immunologic profiles on normal individuals in cancer families. The cytotoxicity assay will be applied to animal systems attempting to relate the cellular and humoral immune response to oncogenic viruses.

Date Contract Initiated: July 12, 1971

CHILDREN'S HOSPITAL OF PHILADELPHIA (NO1-CP3-3272)

Title: The Propagation and Seroepidemiology of Epstein-Barr Virus (EBV)

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Berge Hampar

Objectives: To investigate the relationship between EBV and human cancer. Studies on this contract include: (1) improvement of existing, and development of new techniques for the detection of EBV-related antigens and titration of corresponding antibodies; (2) search for methods to measure cell-mediated immune reactions in EBV-associated diseases, and, if successful, their application to detection of blocking (tumor enhancing) factors; (3) determination of frequencies and titers of antibodies to various EBV-determined antigens in EBV-associated diseases providing prognostic information and support for a causal relation of EBV to given human malignancies.

Major Findings: Collaborative studies in Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) were completed and based on these data new studies were initiated to apply EBV-serology in diagnosis/prognosis of BL, NPC and infectious mononucleosis (IM).

In Burkitt's lymphoma EBV-DNA was detected in nearly all biopsies tested and a direct relationship between anti-EBNA and dermal response to recall antigens was observed in African BL patients. Inverse relationships between anti-EBNA titers of Ghana BL cases and dermal response to Raji cell extracts were found. Out of 14 BL cases among pre-bled children in a a prospestive study initiated by IARC in Uganda, 3 were not associated with EBV. All except one out of 11 EBV-associated BL patients developed EBV-EA antibodies with the emergence of tumor. Since sera from 11 cases collected 5-54 months before BL diagnosis had anti-VCA titers well above the GMT of controls of the same age, it is suggestive that BL is not an immediate result of rare delayed primary infections but a later secondary event in carrier of the virus. A new EBV study initiated in Ghana where BL is endemic had shown that 31 infants had maternal antibody to VCA at one month of age, which declined 2-8 months later, depending upon the initial titers. The longer the maternal antibodies persisted, the later seroconversion tended to occur. The intervals between disappearance of maternal anti-VCA and seroconversion was accompanied by no significant clinical or hematological signs of IM and most minor heterophile antibody responses. Over 90% developed antibodies to the EA-R rather than EA-D as found with IM. Thus perinatal infections may play a role in later development of BL but do not occur due to passive protection and EBV infections in early infancy indeed remain silent as previously suspected.

A cluster of four EBV-associated BL cases was found in young American adults living within 30 miles of each other.

Nasopharyngeal Carcinoma. A five year longitudinal study of Chinese NPC revealed that EBV-serology had a place in the management of this cancer. Rise in EBV antibody titers was noted months in advance of the clinical detection of relapse or metastasis. EBV-DNA or EBNA positive tumor cells were found only in undifferentiated carcinomas of the nasopharynx or the adjacent nasal fossa but not in carcinomas at other sites. A few non-NPC patients presented EBV serology, similar to NPC patients, presenting a critical review of such cases for relatedness of the virus. The contractor has initiated EBV-serological studies in American and Alaskan NPC.

Infectious Mononucleosis (IM). Killer T-cells specific for EBV-transformed cells were found among circulating lymphocytes of acute phase of IM patients but not in late phase (convalescence) or in cases of IM-like illnesses unassociated with EBV. Antibodies responsible for antibody-dependent cellular cytotoxicity arise slowly in IM and react at the levels observed in healthy viral carriers months after onset of illness. A study involving primary EBV infections has been initiated to assess clinical and heterophile antibody responses in early childhood. Studies have also been initiated to investigate fatal or unusual cases of IM to obtain insight into the pathogenesis of IM.

Significance to Biomedical Research and the Program of the Institute: The primary purpose of these studies is to aid in the determination of the etiologic relationships of EBV to certain human malignancies. Fingerprints of EBV have been found in nearly all BL and NPC biopsies. EBV-related serology may serve to detect advancing disease, to provide prognostic information, and to monitor the effectiveness of therapy.

Proposed Course: Continue improvement of existing assays (particularly EBNA) and the development of new assays for the measurement of humoral and cell-mediated immunity to EBV-associated antigens. Conduct continued longitudinal studies on African BL patients to provide further evidence for the prognostic significance of EBV-related antibody patterns and to determine, especially, the relationship of anti-EBNA titers to clinical events. Complete studies on NPC in collaboration with Dr. John Ho in Hong Kong. Determine the relationship of EBV titers and T-cell function in studies on renal transplantations and infectious mononucleosis.

Date Contract Initiated: March 1, 1973. This is a continuation of the Contract PH 43-66-477 initiated February 2, 1966.

DUKE UNIVERSITY (NO1-CP3-3308)

Title: Expression of the RNA Tumor Virus Genome in Animal and Human Malignant Cells

Contractor's Project Director: Dr. Dani P. Bolognesi

Project Officer (NCI): Dr. Peter J. Fischinger

Objectives: (1) To study in detail the properties of structural components of RNA tumor virus particles, particularly those of mammalian RNA tumor viruses. (2) To utilize these materials for preparation of highly specific antisera which can be applied to the analysis of cells for the presence of similar virus gene products. (3) To develop appropriate antisera which can be employed for detection and identification of tumor virus activities in human malignant cells. (4) To use purified structural viral proteins and the corresponding antisera for possible immunological control of viral disease.

Major Findings: Murine model system. Based on the finding that immunization with purified gp71 of Friend murine leukemia virus (MuLV Friend) and the treatment with heterologous antisera raised against the purified glycoproteins can provide protection against MuLV infection and subsequent leukemia development, two other virus systems were tested. The findings are as follows: (1) Prevention of leukemia in AKR mice was achieved when AKR females and their offspring received at birth anti-gp71 IgG. The analysis indicates that antibody specific for the broadly cross-reacting determinants of this virus glycoprotein can be used to control development of spontaneous leukemias mediated by replicating endogenous viruses. (2) Protection against MSV-induced tumors by use of antiviral antisera was also attempted.

Nonproducer (NP) tumors induced with cells transformed by Kirsten and Harvey sarcoma viruses were non-immunogenic. Infection of these cells with cloned MuLV (Friend) rendered them immunogenic, but not against the NP tumors. Results indicated that NP tumors (nonsuperinfected) are not retarded by any serum treatment, while infected tumors are influenced by treatment with homologous anti-MuLV gp71; however, serum dose is critical. Thus,

virus infection was prevented by treatment with antisera against MuLV (Friend) gp71, suggesting that viral antigens served as functional targets for tumor destruction.

Studies using chimpanzee antiserum or gp71 (murine) in passive serum therapy, against Friend leukemia virus (FLV) protected mice against virus-induced disease. The results indicate a close correlation between the development of antiviral humoral immunity and protection against splenomegaly related to serum and spleen infectious virus titers.

The effectiveness of serum therapy on tumor cell challenge and the relevant antigenic specificities were studied. Transfer of protection with immune serum was possible using minute quantities of serum and it appeared to be mouse strain specific. Furthermore, protection against two other mouse tumors, both of which also arose as mammary carcinomas, could be achieved by this procedure.

The data on immune protection against tumors in a murine model suggest that viral antigens play a key role. These data also point out the key role of antibody-instructed macrophages in the killing of tumor cells.

Immunotherapy in cats. Through a subcontract to Cornell University, the information for the mouse immune protection system was applied to the feline system. Treatment of feline leukemia virus (FeLV) infected kittens with homologous and heterologous goat antiserum to Friend leukemia virus (gp71) major glycoprotein prevented establishment of disease in a high percentage of animals. Goat antiserum to disrupted feline leukemia virus had an effect on the development of leukemia in Friend virus-infected mice. These results suggest that the interspecies cross-reactivity between oncornavirus antigens can prevent and control disease induced by oncornaviruses.

It was suggestive that it is possible to suppress chronic viremia in adult cats with appropriate antibody treatments. In this case, goat anti-FeLV IgG was used.

Immunotherapy of FeSV-induced sarcomas in kittens with antiserum to feline leukemia virus indicated that with such treatment the animals survived infection and also showed regression of palpable tumors. Recovery is also possible by use of interspecies antibodies to major glycoproteins.

Virus structure, properties of components biosynthesis and expression. From Friend murine leukemia virus (FLV) two previously unresolved proteins (p15E and p12E) were clearly demonstrated, in addition to gp71, p30, p15C, p12 and p10. FLV p15E, p12E and gp71 were removed when intact virions were digested with bromelain, whereas remaining components were not affected. These studies support the conclusion that gp71, p15E and p12E are situated on the surface of the virion.

The linkage between viral polypeptides suggested that greater than 90% of avian gp85 and gp35 are disulfide-linked as viral glycoprotein complex (VGP). It also indicated that p19 exists as a network of disulfide-linked molecules. In contrast to the avian system, only 10-15% of FLV gp71

was disulfide-linked to p15E in the VGP and the remaining gp71 is loosely attached to the virus, perhaps by a noncovalent interaction with p12E.

Other Studies. One human breast cancer cell line in vitro was established from a lung tumor. These cells grew in nude mice.

Equine infectious anemia virus studies indicate that major non-glycosylated polypeptides of this virus included three components of 25,000, 14,000 and 11,000 daltons molecular weight. Two glycoproteins of 80,000 and 40,000 daltons were also detected. Sera from diseased animals reacted with 25,000 and 80,000 daltons proteins.

Antiserum against avian myeloblastosis virus (AMV) gp85, which cross-reacts extensively with a carbohydrate side chain is specific for AMV and other viruses produced in vivo by hematopoietic cells, but not by viruses that grow in fibroblast cells.

Influence of interferon on type C virus antigen expression was examined. The purified mouse interferon treatment in acute exogenous MuLV infection reduced the amount of associated virus. Under such conditions the whole AKR genome was transcribed in interferon-treated cells.

The amount of virus-specific RNA detected in interferon-treated cells was 1-7-fold lower than the amount of virus-specific RNA present in infected control cells. The synthesis of gp71 glycoprotein was inhibited by 1-5 folds and both cellular and soluble gp71 were affected. These data confirm that interferon inhibits later steps of MuLV replication; the block occurs after transcription of viral mRNA and synthesis of viral-specific gs antigen and major glycoprotein.

Significance to Biomedical Research and the Program of the Institute: Although human leukemias or other tumors are now known to be associated with replicating RNA tumor viruses, one cannot exclude the possibility that virus genes exist in human cancer cells and are expressed as discrete antigens on the cell surface in a fashion similar to that in animal cells. Regardless of whether or not this occurs, there is considerable evidence that many other aspects, particularly, the immunological consequences of animal and human leukemias, are distinctly related. Therefore, an understanding of the immunological mechanisms in the animal models which are of key importance for host defense, coupled with protocols to artifically stimulate those leading to effective prevention and control of the disease, is of value to reach a better understanding of related events in human cancer. Direct application of the principles which apply in the animal to the disease in humans can occur when appropriate human leukemia-specific antigens are identified and characterized, a long-term goal of this research.

Proposed Course: The contractor will determine the immunological response of rodents and cats to exogenous type C oncornavirus infections and use this knowledge to develop immunological preventive and therapeutic measures against oncornaviral-induced leukemia. Surface antigens of human tumor cells which have been found to share some antigenic sites with well-characterized constituents of animal oncornaviruses will be identified and characterized.

Date Contract Initiated: March 1, 1973

DUKE UNIVERSITY (NO1-CP4-3395)

Title: Immunological Studies on the Relationship of Embryonic Antigens and Virus-Associated Antigens

Contractor's Project Director: Dr. Samuel A. Wells

Project Officers (NCI): Dr. David Klein
Dr. Robert Friedman (Assistant)

Objectives: To evaluate the immunological similarity known to exist between fetal cell antigens and the antigens of tumor cells induced by oncogenic and endogenous viruses with emphasis on studies on immunity to Schmidt-Ruppin (SR) virus-induced sarcomas in mice.

Major Findings: The immunological relationship of embryonic antigens to virus-induced tumor antigens was emphasized. In such studies the contractor employed C57Bl fetal antigens to Schmidt-Ruppin-D strain chick leukemia virus, endogenous murine virus and tumor-specific surface antigens. Specifically, the study was to evaluate the relationship by in vivo tumor transplantation resistance and fertility studies by in vitro humoral and cellular cytotoxicity studies by use of immunocytochemistry and microcellular cytotoxicity assay before and after adsorption of the antisera with virus-containing chick and mouse cells. The following conclusions were drawn. The tumors produced in C57Bl/6 mice by Schmidt-Ruppin-RSV contained the RSV genome and expressed variable quantities of MuLV. The in vivo immunogenicity measured by ability to induce tumor transplantation resistance is directly proportional to MuLV. The ability of these tumor lines to induce cytotoxic antibodies was inversely proportional to MuLV content.

Fetal cell immunization could produce tumor transplantation resistance if the tumor cell inoculum was titered.

Neither primiparous nor multiparous mice possessed any natural resistance to tumor transplantation. Multiparous animals also possess in vitro cytotoxic antisera.

Cytotoxic antisera can be produced by fetal antigen or tumor antigen immunization. In case of fetal immune antisera, the class of antibody active in in vitro cytotoxicity appeared to be an IgM. Absorption studies suggest that there is no evidence of any RSV antigens in the in vitro reactions. Furthermore, these studies suggest that syngeneic fetal cells possessed all the antigenic components present on Friend and Gross MuLV-infected cells, but the converse was not true.

Significance to Biomedical Research and the Program of the Institute: The hypothesis that depressed host genes are responsible for oncogenesis

is being actively studied by several groups in cancer-related programs. The relationship between fetal antigens and virus-associated antigens, particularly those associated with endogenous virus expression, is an important issue in relation to the question of etiology as well as to programs concerned with prevention and control of virus-associated cancers. To understand better the mechanism of neoplastic transformation, it is essential to develop the tools to distinguish between host gene re-expression and new viral gene products.

Proposed Course: This contract terminated November 30, 1977

Date Contract Initiated: June 28, 1974

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CP5-3570)

Title: Demonstration of Tumor-specific Transplantation Antigens in Animal and Human Tumors with the Microcytotoxicity Assay

Contractor's Project Directors: Dr. Karl Erik Hellstrom
Dr. Ingegerd Hellstrom

Project Officer (NCI): Dr. David Klein

Objectives: (1) To continue studies on immune responses against viral and putative embryonic antigens in chemically-induced tumors, using in vivo and in vitro assay techniques. (2) To develop methods of immunological prevention and of monitoring the development of chemical tumors. (3) To study "natural immunity" against chemically and virus-induced tumors. (4) To study the nature of antigen-antibody complexes in the kidneys of multiparous mice. (5) To study methods of in vitro sensitization of lymphocytes against viral proteins. (6) To continue studies on the cellular and humoral immune responses of human subjects against tumor-associated antigens, including those of healthy individuals, tumor patients, and in relatives and close contacts of tumor patients. In particular, to investigate "natural immunity" to tumors in man.

Major Findings: In studying the incidence of murine leukemia virus (MuLV) expression in BALB/c MCA (methylcholanthrene)-induced fibrosarcomas, 15 primary and transplanted sarcomas were studied. The findings can be summarized as follows: Expression of MuLV in vivo was assessed by a group-specific RIA for p30. Most primary tumors had p30 levels similar to those found in normal tissue. On the other hand transplanted tumors repeatedly contained higher levels of p30.

Sarcomas possessing low levels of p30 maintained in cell culture, when tested for MuLV expression, lacked p30 and reverse transcriptase activity in the culture medium, whereas supernatants of cell cultures from high p30 sarcomas (in vivo) produced MuLV produced MuLV in variable amounts.

Sarcoma cells were tested for MuLV antigens by group-specific antisera against p30 and gp70. Both an I^{125}-labeled protein A binding assay and complement-dependent cytotoxicity assays demonstrated gp70 and in some cases gp30 on the surface of those tumor cells that had been typed as virus producers. Such identification was not found on cells that did not produce virus. Hyperimmune sera from sarcoma-bearing animals were tested for antibodies to cell surface antigens. MuLV antigens, possibly gp70, can elicit higher titers of antiviral antibodies. No evidence for production of antibodies to unique TSTA was found in such sera.

Transplantation tests indicated that each of the sarcomas expressed unique TSTA; however, cross-reactivity between different tumors was also observed, suggesting that it may be related to MuLV expression.

Significance to Biomedical Research and the Program of the Institute: Host immune functions are major factors in the control of disease processes, including overt neoplasia. This project was initiated to obtain increased understanding of the immune system in relation to susceptibility and resistance to cancer.

Proposed Course: This contract will terminate December 31, 1978.

HEBREW UNIVERSITY (NO1-CP3-3342)

Title: A Multidisciplinary Study of Hodgkin's Disease in Israel

Contractor's Project Director: Dr. Natan Goldblum

Project Officer (NCI): Dr. Paul Levine

Objectives: To investigate the interrelationship between environmental and genetic factors in the etiology, pathogenesis and spread of Hodgkin's disease (HD) in Israel and, in particular: (1) to evaluate clustering among social contacts, in space and time, and to develop methods for the study of clustering; (2) to evaluate HD in immigrant and native-born Israelis, including studies of HLA typing, and antibody levels to Epstein-Barr virus (EBV) and other viruses; (3) to review the histological sections of all malignant lymphomas diagnosed throughout Israel during 1964-1972 so as to provide basic information necessary for the immuno-epidemiologic studies; and (4) to establish laboratory assays relevant to an understanding of the pathogenesis of malignant lymphoma.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: This project permits a comprehensive study of all lymphoma cases arising in a large, well-defined population, with comparable measurement of virus-exposure, immunologic status, and genetic background on a control group. Mortality data, an unreliable but useful indicator of environmental

identified 5-15% of children in ages of 4-11 years who possessed high VCA antibody (close to titers observed in BL patients), suggesting that they are at 30-fold higher risk in developing BL.

Time space clustering was further observed in the West Nile District (1961-1975), indicating the involvement of an environmental agent in causation of BL. A similar analysis of BL cases occurring in North Mara, Tanzania, however, failed to show time space clustering of BL.

The application of various combined agents was successful for improving EA positive cells in Raji cell cultures. Moreover, a micro ELISA test has been developed for detection of EBV-EA antibodies. Similar methods are employed to develop a test for EBV-VCA antibody testing, which may also be sensitive and economical.

Nasopharyngeal Carcinoma (NPC). Further studies of genetic markers showed that in addition to A-2 B-Sin 2 HLA haplotype, the A-blank B-W17 HLA profile was found with a higher frequency among newly diagnosed NPC Chinese patients. Within the same group of patients, an association between survival rate and HLA could be demonstrated. Lack of correlation between HLA profile and either non-specific cell-mediated immunity or EBV-titers was found. Furthermore, IgA specific and anti-EBV antibodies were found in serum and saliva of NPC patients, which appears to be a unique feature. A control population lacked this antibody. Three cases of NPC were diagnosed among the people who were bled in the sero-epidemiological survey in Hong Kong from 1972-1974. These cases showed anti-IgA to EBV-VCA which were elevated between pre-bled and post-tumor serum samples, suggesting diagnostic implication of this antibody for occult tumors of the head and neck region. These sera showed no elevation of antibody titers to IgG-VCA and IgG-EA. A greater blastogenic response and release of macrophage inhibitory factor (MIF) were induced by membrane extracts of Raji cells and NPC pooled biopsies in cultures of lymphocytes from NPC patients than in controls.

In vitro attempts to infect normal nasopharyngeal epithelium with EBV were continued. Short-term cultures of epithelial cells from apparently normal mucosa of NPC patients, when infected with EBV, led to synthesis of the EBNA.

The search for genetic markers with NPC in Tunisia and Singapore was continued.

A case control study of NPC in Hong Kong showed that the risk of getting NPC is positively associated with such factors as previous disease of the ear and nose and certain aspects of the traditional Chinese life style.

Significance to Biomedical Research and the Program of the Institute: To date, EBV is the only naturally-occurring virus strongly suspected of an etiologic role in human cancer. The research under this contract should help elucidate the role of EBV in two human tumors, Burkitt's lymphoma and nasopharyngeal carcinoma. Initial tests on individuals of various ethnic groups shows that there are significant differences among populations at different risk of NPC. The results obtained by blood genetic typing,

together with the seroepidemiological results, may provide the means for detecting high risk groups among a normal population for NPC.

Proposed Course: In Africa, case detection will continue to have high priority in order to determine the relationship of EBV antibodies to subsequent appearance of disease. The nasopharyngeal carcinoma program will be continued to define the genetic factors relating to disease. Immunologic and virologic studies will be conducted to determine whether or not the EBV isolated from Burkitt lymphoma cases is identical to that isolated from NPC.

Date Contract Initiated: June 11, 1970

LITTON BIONETICS, INC. (NO1-CP4-3333)

Title: Immunological Assays for DNA and RNA Viruses

Contractor's Project Director: Dr. David Fuccillo

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To analyze humoral and cellular immune functions in normal individuals and cancer patients against Epstein-Barr virus (EBV) and to apply immunologic assays measuring EBV immunity to the control of human cancers, primarily Burkitt's lymphoma and nasopharyngeal carcinoma. To support other investigators in the cancer field by means of expertise in assays and/or distribution of materials and reagents collected and stored as a result of the contract.

Major Finding: An indirect immunoperoxidase assay developed for EBV-serology was compared with indirect immunofluorescence now being employed routinely. The immunoperoxidase test was sensitive at levels where the FA test could not detect any reactivity in EBV sera. Moreover, the test was specific, reproducible and economical, since it does not require a FA microscope, and permanent records of slides can be maintained.

NPC patients were tested by antibody-dependent lymphocyte cytotoxicity (ADLC). The results suggested that this test may be of prognostic value.

A direct leukocyte migration inhibition (LMI) assay was developed for studying CMI responses to EBV antigens. Large numbers of clinically normal blood donors were also used to further assess the specificity of the assays (agarose droplet and capillary tube assays). The agarose plate method was found to be suitable to conduct CMI studies on patients from world-wide sources since this test can use frozen patient-derived materials.

Several EBV-antigens were used in the LMI assay (transforming and non-transforming virus). Virus preparations from B95-8 and P_3HRI appears to be the best antigen to demonstrate positive LMI in known EBV-seropositive

individuals. An increase in CMI values was found from severe infectious mononucleosis patients as compared to those with asymptomatic disease.

The fluorescent antibody (FA) evaluation of existing lymphoblastoid cell lines showed a complex package of antigens which are specifically related to EBV infections.

In the support service part of the contract, the contractor received and distributed more than 5,000 sera and tissue specimens during the year in the U.S. and abroad. The contractor also maintained a computer information-base for collaborative services and provided serological assays.

Significance to Biomedical Research and the Program of the Institute: Epstein-Barr virus is oncogenic in some monkey species, is ubiquitous in the human population, and is known to cause at least two clinical syndromes, infectious mononucleosis and abacterial tonsillitis. There is increasing evidence that it is involved in the cause of Burkitt's lymphoma, naso-pharyngeal carcinoma, and Guillaine-Barre, an infectious neurologic disease. It is of great importance to acquire information on the different factors that may influence the outcome of EBV infection, particularly development of cancer.

Proposed Course: This contract will be phased out within the next year.

Date Contract Initiated: June 3, 1974

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP4-3222)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-immunologic Methods

Contractor's Project Directors: Dr. Paul H. Black
Dr. Martin S. Hirsch

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: To determine the effects of human interferon in the prophylaxis of virus infections in immunosuppressed kidney transplant patients. To study the cellular reactivity of normal humans and of patients with neoplastic and other disorders to primate oncornavirus antigens.

Major Findings: Continued study of the efficacy of interferon from human leukocytes in patients enrolled in a double blind, placebo-controlled study initiated in renal transplant recipients was evidenced by enrolling more patients and by introducing modification in protocols. These patients were closely monitored for viral infections (CMV, BK, Varicella Zoster) as well as rejection episodes and signs of toxicity. Since the introduction of these modifications, bone marrow toxicity has not been a problem and no significant immediate toxic effects (sore arm and fever) have been noted. The complete data from this study will be evaluated at a later date.

However, of these patients 16 have thus far excreted herpes simplex virus from the throat, 15 have shed CMV in urine or in the throat. Four patients have viremia. Six patients have a febrile-leukopenic episode associated with CMV. Many rejection episodes of varying degree have been observed. As the identification of patients is still under code, differences between interferon and placebo groups with regard to viral excretion, clinical infections or rejections can not be evaluated.

Since BK papovavirus (BKV) is frequently detected in kidney transplant patients, attempts were made to infect lymphoid and myeloid cell lines, as well as peripheral blood leukocytes with BK virus. The infected cells will be followed for evidence of lytic infection and viral transformation.

Cell-mediated and humoral immune responsiveness in 6 out of 7 women was demonstrated against antigens associated with primate type C oncornaviruses (e.g. simian sarcoma virus) in humans. Maximum reactivity was observed against baboon endogenous virus (BEV) during pregnancy. The activity could be blocked by purified BEV. Primate type C (SSV/SSAV) viruses were grown in human myeloblastoid and B and T lymphoblastoid cell lines. Productive, non-lytic infections were established in all three types of cells with these viruses and these cells have been in culture over three months. Further studies will be carried out to study the interaction of these viruses with normal leukocytes.

Significance to Biomedical Research and the Program of the Institute: Although cancer risk in organ transplant patients is high, it is doubtful whether the small number of patients receiving interferon prophylaxis would provide significant differences in the incidence of cancer. The studies on the inhibition of virus infection in these immunosuppressed patients should provid valuable data on interferon prophylaxis, which may later be more directly applicable to cancer prophylaxis and therapy. The information obtained on human cellular reactivity to simian sarcoma viruses is pertinent since it supports the viral relationships to human cancers.

Proposed Course: The current clinical studies will be continued according to the protocols already established, with appropriate modifications, as these seem clinically indicated.

Date Contract Initiated: September 15, 1971

MERCK AND COMPANY, INC. (NO1-CP1-2059)

Title: Research on Oncogenic and Potentially Oncogenic Viruses, Virus Production and Vaccine Development

Contractor's Project Directors: Dr. Maurice Hilleman
Dr. Vivian Larson

Project Officer (NCI): Dr. Michael Chirigos

Objectives: The overall objective is to determine the practical feasibility for immunologic prevention of virus-induced cancer applicable for use in humans. The specific objectives are: (1) Project A - to develop a herpes simplex type 2 (HSV2) subunit vaccine suitable for prophylactic trials in man against genital herpetic disease and against possible subsequent herpesvirus-induced cancer; and (2) Project B - to evolve type C virus vaccine technology for future application in preparation of leukemia virus vaccines suitable for use in humans by developing a safe and effective vaccine against leukemia in the cat model system.

Major Findings: Both vaccine development programs were brought to the stage of preparation of experimental virus glycoprotein vaccine production and evaluation in animals.

Herpes Simplex (HSV) Type 2 Vaccine Studies. Five glycoprotein-rich antigens were extracted from HSV-type 2 infected chick embryo cell cultures. The solubilization of these antigens was attempted by Triton X-100 and NaCl, followed by DEAE-cellulose and hydroxilapatite chromatography. Only one antigen is considered for clinical use, mainly as an immunogen for vaccine studies.

This antigen was composed of five viral-directed glycoproteins and polypeptides. This antigen was immunogenic (has neutralizing and cell-mediated activities) in guinea pigs, cats and marmosets. An alum-adsorbed vaccine of this antigen is protective in mice against HSV-2-induced paralysis and death. The antigen was also found to be free of infectious virus, virions, and double-stranded DNA. One of the other four antigens was eliminated as a potential vaccine since high DNA contents were detected. Of the three remaining, two are being tested for potential use. Attempts were also made to further purify these antigens.

Feline leukemia virus (FeLV) vaccine studies. An FeLV-strain-1 and 75,000 dalton viral envelope glycoprotein (gp75) were tested as a possible vaccine against FeLV in guinea pigs and cats. The inoculum included 760 mg of purified FeLV, 9.5 mg of purified gp75. These products were combined with adjuvant 65 or alum. The animals were analyzed for seroconversion neutralizing antibody, cytotoxic antibody and lymphocyte blastogenesis. Aqueous vaccine induced only 50% seroconversion. In contrast immunization of SPF cats with twice the dose level (40µg) induced a significantly lesser degree of FeLV immunity when given in alum or Freund's adjuvant. Cats immunized with 40µg in alum adsorbed or Freund's adjuvant developed antibody 10-15 folds below comparable immunized guinea pigs and little or no neutralizing antibody or cytotoxic antibody was found. Cellular immunity was detected in half of alum or adjuvant gp75 vaccinated cats. Normal age resistance to FeLV infection prevented adequate evaluation of the protective efficacy of gp75 vaccine against FeLV challenge.

Significance to Biomedical Research and the Program of the Institute: If viruses are proven to cause some human cancers, a vaccine may effectively prevent or minimize infection or pre-sensitize the host to reject antigens

expressed during the earliest stages of tumor development. Since live attenuated or killed virus vaccines for potentially oncogenic viruses would not be acceptable for human use due to the danger of transfer of functional viral-genetic material, this project was initiated to determine whether vaccines to purified viral antigens acceptable for use in humans were of practical value.

Proposed Course: This contract will terminate August 31, 1978.

Date Contract Initiated: March 1, 1971

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP3-3248)

Title: Immunoprevention of Spontaneously-occurring Neoplasms

Contractor's Project Director: Dr. Marshall Dinowitz

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To examine immunoprevention of spontaneously-occurring tumors in laboratory mice by the use of viral and/or cellular vaccines. This included studies on the parameters involved in spontaneous neoplasia including incidence, progression and histologic types of tumors, endogenous type C virus expression, and natural and induced humoral and cellular immunity to viral and nonviral tumor antigens.

Major Findings: Induction of active immunity or transfer of passive immunity to endogenous type C virus showed reduction of x-ray induced leukemias in C57Bl mice. In these studies, active immunity was established by immunization with inactivated R-MuLV, and passive immunity was induced by goat anti-G-MuLV immune IgG. Both of these treatments induced circulating antibody to AKR-gp70 through the latent period of tumor development. Both treatments also resulted in reduced tumor incidence and in apparent immunoselection against virus-positive tumors.

Resistance to virus-free syngeneic tumor transplantation in BALB/c mice was observed after immunization with type C virus-infected homologous tumor cells.

The immunogen from homologous tumor cells, having high Rauscher leukemia virus, was shown to be immunogenic in adult mice, since it provided tumor protection and no leukemogenesis was detectable. Splenic lymphocytes from immune animals were shown to be cytotoxic in vitro and by adoptive transfer experiments were shown to prevent tumors in vivo.

A study was conducted to find the action of structural antigens on the surface of infected cells. In these experiments, antisera raised previously against major structural proteins of R-MuLV cell lines

infected with ecotropic murine leukemia virus of the FMR and AKR type and the two known classes of xenotropic murine leukemia virus were employed. By use of a complement dependent cytotoxicity assay, virus specific cytolytic activity was observed with anti-R-gp70 and anti R-p12. Immunoassays showed gp70 and p15(E) on the viral surface, whereas p12 and p30 were not.

Experiments involving natural occurrence of tumors in mouse strains differing in their xenotropic and ecotropic endogenous viruses were completed. The results showed a low level but ubiquitous presence of the xenotropic p12 in all naturally-occurring tumors of these two strains of mice. Since in cytolytic reaction p12 is an effective target antigen as shown previously, this viral protein could be of critical value as a transplantation antigen.

Significance to Biomedical Research and the Program of the Institute: This contract contributes to an understanding of the genetic and epigenetic relationship of endogenous type C viruses to the host's naturally-occurring tumors and explores immunologic approaches to tumor control.

Proposed Course: Investigate measures for immunoprevention of spontaneously-occurring tumors in the BALB/c mouse with viral and cellular vaccines; attempt immunoprevention of chemically-induced tumors in BALB/c and C57Bl mice with vaccines produced from cells containing type C virus; and study immunologic factors in C57Bl mice with radiation-induced tumors. Immune response potential will be studied in aging mice in conjunction with endogenous virus expression. The complex interaction of humoral and cellular immunity and their respective roles in spontaneous neoplasia occurring with advanced age will be studied.

Date Contract Initiated: November 15, 1961

MOUNT SINAI SCHOOL OF MEDICINE AND HOSPITAL (NO1-CP4-3225)

Title: Stimulation of Immunity to Virus-Associated and Tumor-Associated Antigens in Mouse Systems

Contractor's Project Director: Dr. J. George Bekesi

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To evaluate chemoimmunotherapeutic measures in tumor-bearing animals using neuraminidase-treated cells and viral inhibitors to determine the best means for controlling the oncogenic virus and the tumor. (2) To examine the virus titer and specific anti-virus immunity in animals undergoing chemoimmunotherapy and in untreated controls. (3) To transfer the knowledge gained from these animal studies to clinical applications against human leukemia.

Major Findings: Mouse Leukemia Studies. The antibodies in the sera of AKR mice, which react with neuraminidase-treated leukemic cells can be considered to be autoantibodies, since they can be completely absorbed not only by leukemic cells but also by normal AKR lymphoid cells. The development of spontaneous leukemia in AKR mice was accompanied by the disappearance of autoantibodies to thymus cells while there was only slight reduction at the level of autoantibodies to neuraminidase-treated tumor cells. No autoantibodies could be detected in AKR mice having spontaneous leukemia. In fact, the serum from the leukemic mice was found to inhibit cytotoxicity of the sera of normal AKR mice. Mice having spontaneous leukemia, with no autoantibodies in their sera, were treated with cytoxan. After this treatment, half of the mice had thymic autoantibodies. Thus, these studies suggest that presence or absence of thymic antibodies in the serum of leukemic AKR mice undergoing therapy may be an indication of the load of leukemic cells to which the animals are exposed.

Clinical Leukemia Studies: Use of immunotherapy with neuraminidase treated allogenic myeloblasts in AML was continued. Fifty-eight patients were divided in two groups following remission and the use of cytosine arabinoside and daunorubicin were evaluated. Of patients that received immunotherapy, in 30/58 the remission duration on chemotherapy alone was 44 weeks, whereas those receiving combined immunochemotherapy had not yet reached the median at 110 weeks. Thus the difference in the two groups appeared to be highly significant. The comparison in these two groups by *in vitro* and *in vivo* immunodiagnostic tests also suggested that the combined therapy patients demonstrated restoration of normal immunocompetence in comparison to those who received chemotherapy alone.

Mammary Tumor Studies. Antigens were solubilized from MTV-induced tumors in BALB/CFC$_3$H GRGL mice. These solubilized antigens showed biological activity by inducing lymphoblastogenesis in previously sensitized splenic lymphocytes from syngeneic animals. The blastogenesis, however, was abrogated by serum from tumor bearing animals. AMMT neutralized complement dependent rabbit anti-ML(v) antibody activity. One component of AMMT possessed an electrophoretic mobility pattern identical to that of ML(v) antigen.

Treatment of Herpesvirus Saimiri (HVS) Induced Tumors. To use a nonhuman primate model for evaluation of antiviral drugs against malignant lymphoma /leukemia, HVS was inoculated in owl monkeys to induce such tumors. Phosphonoacetic acid (PAA), a DNA virus inhibitor was found to be effective at nontoxic concentrations in completely inhibiting HVS production *in vitro*. Furthermore PAA was highly effective even when added to cultures at 124 post HVS infection. Circulating PAA was found in the blood of mice, rabbits and monkeys after oral or subcutaneous administration. A dose of PAA was calculated (150-200 mg kg/day) to be used in the treatment of HSV lymphoma/leukemia in owl monkeys. Data suggest that this dose is well tolerated by the animal and it also slowly increases in blood. PAA reaches its maximum level *in vivo* in blood at approximately 80 $\mu g\ ml^{-1}$ in 4 days infusion. The *in vivo* treatment of HVS tumors will be continued with suggested dose levels. Use of human interferon alone or in combination with PAA and other antiviral agents is planned.

Significance to Biomedical Research and the Program of the Institute: Evidence suggests that in acute leukemia, breast cancer, and Burkitt's lymphoma, late relapse may actually be disease reinduction due to persistence of the factors initially associated with the disease. If there is a viral etiology for these three tumors, it is important to obtain methods of permanently controlling the virus while chemotherapy or immunotherapy are used to control the tumor itself. Successful development of treatment protocols in animals with leukemia, breast cancer, and lymphoma are an important first step in curing these forms of human cancer.

Proposed Course: The activities described will be continued at the current level.

Date Contract Initiated: August 6, 1973

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CP5-3571)

Title: Immunobiologic Response of the Cat to Feline Oncornavirus

Contractor's Project Director: Dr. Richard G. Olsen

Project Officer (NCI): Dr. Alfred Hellman

Objectives: Studies concentrated on development of a regimen for inducing immunity to feline oncornavirus and virus-specific tumor antigens. Specific objectives are: (1) To solubilize and characterize the feline oncornavirus-associated cell membrane antigen (FOCMA); (2) to prepare purified feline leukemia virus (FeLV) envelope glycoproteins (gp 69/71); (3) to evaluate the vaccine potential of FOCMA and FeLV gp 69/71 for the prevention of viremia and tumorigenesis in specific pathogen-free cats; (4) to evaluate the lymphocyte blast transformation responses to defined FOCMA and FeLV gp 69/71 antigens in vaccinated cats and cats infected with virulent feline oncornaviruses; (5) to determine whether a chemical carcinogen, methylnitrosourea, alters the age-related susceptibility of cats to feline oncornavirus disease; (6) to determine whether chemical carcinogens alter the immunobiologic responses of cats to feline oncornaviruses; (7) to investigate further the relationship of age, virus strain, route of infection, and other immunologic or cocarcinogenic factors which influence the susceptibility of cats to feline oncornaviruses; (8) to evaluate the mixed lymphocyte-tumor cell assay using sensitized feline peripheral blood lymphocytes and inactivated lymphoblastoid cells to detect tumor-associated antigen blast transformation.

Major Findings: A technique was developed to obtain larger yields of soluble FOCMA from culture media (free of serum). FOCMA was found to elute as approximately 100,000 molecular weight component on gel filtration columns. Precipitation of radiolabeled FOCMA with appropriate primary and secondary reagents suggested that there may be two different components (approximately 20,000 and 30,000 molecular weight).

Immunization of kittens with partially purified FOCMA induced specific antibody as observed by membrane fluorescence staining on S+L mink infected cells (FOCMA positive and virus negative). A 75-85,000 molecular weight component on FL-74 cell surface reacted with FOCMA antisera.

Active and passive immunity to FeLV demonstrated that inactivated purified FeLV did not induce immunity in pregnant cats or in their sucklings. These data confirm the poor immunogenicity of nonreplicating FeLV vaccine and are consistent with data regarding FeLV-mediated immunologic abrogation studies. In the in vitro assays, killed FeLV and FeLV-p15 were shown to abrogate feline lymphocyte function (i.e., inhibition of LBT and response to con A). Other FeLV proteins (i.e., FeLV-p30 and FeLV-gp70) did not induce responses to killed FeLV and FeLV-p15. The abrogation appears to be a specific suppressive effect of viral protein on feline lymphocytes. The immunization of kittens with killed FeLV or FeLV-p15 significantly interfered with their ability to immunologically respond to FOCMA.

The effect of surgical removal of thymus from kittens prior to FeLV infection did not alter the susceptibility to FeLV infection or transformation of T-cells. In these animals T-cell tumors still developed, but FeLV group-specific antigen (gsa) appeared in leukocyte precursor in bone marrow before gsa-negative cats have infectious FeLV in their plasma.

Significance to Biomedical Research and the Program of the Institute: Prophylactic immunization protocols in model systems, such as those carried out under this contract, evaluate prospects for control of cancer induced by horizontally transmitted viruses. The information obtained in such work may be applicable to prophylaxis against other animal oncornaviruses and may possibly have particular future value in control of some human neoplastic diseases.

Proposed Course: This contract terminates September 15, 1978.

Date Contract Initiated: June 25, 1965

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP3-3219)

Title: Studies of Tumor Viruses in Small Primates

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officer (NCI): Dr. Dharam V. Ablashi

Objectives: (1) To maintain a marmoset breeding colony. (2) To study responses of these primates to viruses known or suspected to produce cancers in humans of in animals.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: Restrictions in the host range of viruses require the availability of a primate animal for the study of viruses associated with neoplasia in humans. The marmoset has proven to be most responsive to the oncogenic activity of known tumor viruses. This species is particularly valuable for the study of herpesvirus strains that induce malignant transformation. Viruses of the herpes group are widely distributed among animal species, and infections by a number of these agents are known to result in the development of malignant tumors in the host. It is most important to expand our knowledge of herpesviruses with respect to cancer in primate animals and of their potential for oncogenicity in humans.

Proposed Course: This contract was terminated on March 31, 1978).

Date Contract Initiated: March 15, 1962

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF, AND CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CP5-3500)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
Dr. Brian E. Henderson (USC)
Dr. Robert M. McAllister (Children's Hosp.)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To mount a multifaceted, highly interrelated program designed to determine the roles of viruses, physical and chemical carcinogens, and other factors in the etiology of human and animal cancer in a natural urban ecology. These studies are carried out at USC School of Medicine and at the Children's Hospital of Los Angeles.

Viral Studies: Human, animal pet and fetal animal cancer and fetal materials are under intensive study for RNA tumor virus genetic expression, using all the modern in vitro as well as in vivo test systems. Extensive field studies and procurement efforts provide large numbers of tissues derived from cancer patients, genetically defective individuals, and spontaneous and therapeutic abortions. These materials were used for in vitro and in vivo biological studies and were subjected to serological, immunological, biochemical and electron microscopic analyses designed to detect, isolate and characterize RNA viruses and virus-specific antigens associated with naturally-occurring animal and human neoplasms.

Epidemiological Studies: This program provided, through hospital record surveys and community questionnaire surveys, up-to-date information of the natural occurrence of human cancer as it may be influenced by genetic, viral, environmental or other factors, including exposure to

variable smog components in differing ecological areas of Los Angeles County, industrial and household carcinogens, and pets with and without cancer. Other factors such as occupation, aging, genetic defects, smoking, hormone therapy, and immunosuppressants were studied using classical epidemiological methods combined with virological and serological surveillance.

Environmental Studies: This program was concerned with monitoring focal environmental areas for levels of carcinogens and other air pollutants. Materials collected were characterized and supplied to laboratories at USC, as well as to NCI and other VCP contract programs; e.g., Contract NO1-CP4-3240, for studies to determine the carcinogenic activities of such pollutants in tissue culture and in animals.

Major Findings: Studies in wild mice, mink cells, rat embryo cells and cats: MuLV is acquired exclusively by maternal congenital infection in lymphoma-paralysis bearing LC wild mice. Passive immunization of newborn LC mice with heterologous antiviral antisera reduced the prevalence of detectable viremia at weanling age by 20%. The presence of infectious virus and lymphoma and paralysis could be completely eliminated by selectively breeding nonviremic LC females. Amphotropic virus was found to be the most prevalent virus class in wild mice and so far has been isolated only from wild mice. Ecotropic MuLV was recovered from wild mice mainly in association with the lower limb paralytic disease. Both amphotropic and ecotropic virus clones were transmissable to NIH Swiss mice and these animals developed lymphoma but only with the ecotropic clone paralysis it accompanied.

An endogenous xenotropic mink virus was isolated from CCL-64 mink lung cells.

In the endogenous RaLV shedding rat embryo cells system, the high predictable transformation event was prevented by treatment of the virus-producing, but as yet nontransformed cells with a single inoculum of specific RaLV goat immunoglobulin.

In the domestic cat, expression of endogenous RD-114 viral genome at transcriptional and translational (p30) levels was enhanced without apparent virus production in spontaneous lymphomas, sarcomas and carcinomas regardless of the FeLV status. Most lymphoma, carcinomas, and sarcomas of older cats were negative for FeLV RNA, p30 and infectious virus.

FOCMA antibody absorption data suggested that FOCMA is common in cat tumors where expression is independent of FeLV/FSV infection.

Environmental Studies: Crude extracts of photochemical smog from Los Angeles have shown a mutagenic effect upon Ames tester bacteria. The main source of mutagenicity was in the most acidic fraction rather than in the polycytic aromatic hydrocarbon class.

Epidemiological Studies: Poor cellular immunity with inverse ratios to EBV antibody titers were found in healthy members of multiple lymphoma

families. Increase in incidences in lung cancer reported previously suggested that it may be associated with occupational hazards rather than residential air pollution.

Elevated levels of certain hormones (plasma estrogen and prolectin) were considered an important factor in the etiology of breast cancer.

A negative correlation was found between Hodgkin's disease and time-space clustering.

Biochemical Studies: The sequence organization of RD-114 provirus in infected RD cells is probably not random and endogenous RD-114 viral-specific sequences in cat cell chromosomal DNA are integrated covalently bound.

Infective DNA can be detected only in those domestic cat or baboon cells that are releasing endogenous virus or dog cells releasing endogenous type C virus.

A medulloblastoma cell line was established and characterized.

Significance to Biomedical Research and the Program of the Institute: This program searches for causes of human, pet and other animal cancers in a natural ecology, utilizing (1) experimental animal systems, (2) basic viral and chemical carcinogenesis studies, and (3) epidemiological profiles of cancer incidence in humans and animals in the Los Angeles area in relation to exposure to environmental carcinogens.

Proposed Course: This contract terminates September 15, 1978.

Date Contract Initiated: June 26, 1968

SUMMARY REPORT

C. 1. b. (3) COCARCINOGENESIS STUDIES SECTION

October 1, 1977 through September 30, 1978

There is strong evidence that many environmental carcinogens (biological, chemical, or physical) produce an increased risk of tumor development. Although the initiating transformation event may be a "single hit" phenomenon, evidence suggests a multifactorial etiology mediated by the activation of latent viruses or endogenous viral genes. To further understand the mechanisms of carcinogenesis, the Cocarcinogenesis Studies Section (CSS) initiated studies on the nature and the effects of interactions of environmental carcinogens with cancer viruses and/or intracellular viral genetic information.

A. Systems Development. The development of reliable, sensitive standardized in vitro cell-virus transformation assay systems for use as research tools is perceived as being of fundamental importance since such systems must be available before mechanistic studies can be initiated. Emphasis is being placed on the development and use of primate-derived cell cultures in an attempt to establish assay systems directly applicable to human carcinogenesis. (1) A cocultivation system was developed consisting of islands of human skin epithelial cells surrounded by fibroblasts of human origin. Morphological transformation was achieved after treatment with carcinogen N-methylnitroso-N-nitro-guanidine (MNNG) alone as well as with MNNG in the presence of chronically infected baboon endogenous virus (BaEV). The morphology of the transformed cells was more striking in the presence of the BaEV infected cells. (2) One of the most exciting developments in this area was the observation that mutations could be induced in human cells by exposure to the environmental carcinogen 3-methylcholanthrene (MCA). Both ouabain and 6-thioguanine epithelial cell mutants were isolated after exposure to MCA. (3) Flat morphological revertant clones were derived from human osteosarcoma cells transformed by Kirsten murine sarcoma virus (KiMSV). These nontransformed cells were shown to lack the KiMSV genome, but supported murine leukemia virus growth, showed enhanced sensitivity to MSV superinfection, and were readily transformed by 3MC and 7, 12-dimethylbenz(a)anthracene (DMBA).

B. Establishment of Cofactorial Interactions and Study of Mechanisms of Transformation. Previous reports indicated that only those cells that spontaneously produced endogenous rat leukemia virus (RaLV) or were exogenously infected with this virus could be transformed by chemicals. (1) An important finding was the reproducible rescue of src sequences into a rapidly transforming virus from chemically transformed rat cell. This was the first time that the endogenous src genes have been rescued in vitro in the form of stable sarcoma viruses from tumor cells in which no virus had ever been exogenously introduced. The isolation of this virus will aid in understanding the biological significance of src genes particularly as they may represent the most basic common denominator

for cell transformation from diverse environmental factors. (2) Retroviruses were isolated from tumor cell cultures derived from ^{90}Sr-induced osteosarcomas of CF #1 and X/Gl mice. Nucleic acid hybridization techniques were utilized to determine that the genome of these viruses differed significantly from that of Moloney sarcoma and Rauscher leukemia viruses. (3) A progene was identified which was prerequisite for causing leukemia in AKR mice exposed to MCA by skin painting. (4) Host genetic control of ecotropic and xenotropic murine viruses was found to play a definitive role in spontaneous occurrence of leukemia. (5) Acquisition of herpesvirus genes into the mouse cell genome was enhanced during excision and repair of carcinogen (NMU) damage. Enhancement of adenovirus transformation occurred during DNA repair synthesis of NMU-treated rat embryo fibroblasts. (6) Several strains of fetal baboon cells were found to express baboon endogenous virus (BaEV) p28 following treatment with x-irradiation and benz(a)pyrene diol epoxide. (7) Exposure of human skin-muscle or lung cells persistently infected with human cytomegalovirus to chemical carcinogens has demonstrated that certain chemicals will induce cytopathology and productive viral replication; others will cause morphological alteration of the cells.

C. Prevention. It is apparent that a number of intrinsic (host) factors and extrinsic (environmental) factors interact to control virus expression and malignant transformation of mammalian cells in vitro and in vivo. Assuming that viruses are cofactorially involved in chemical carcinogenesis, it has been postulated that the suppression of virus production or viral gene expression may result in suppression of carcinogen-induced neoplasia. In the past few years, several lines of evidence have been developed which lend credence to the possibility of controlling spontaneous or carcinogen-induced neoplasia through the use of active or passive immunization with virus-specific products.

The Fischer rat embryo cell lines have been used extensively to study potential anti-cancer products including viral vaccines and virus-specific antibody, interferon and viral inhibitors. It was recently shown that anti-RaLV IgG added two or three days prior to treatment of cells with 4-nitroquinidine oxide (4-NQO) protects Fischer rat embryo F1706 cells from phenotypic transformation and oncogenicity for the newborn rat. The addition of IgG at the same time as or after treatment with 4-NQO exerted no protective effect. Preliminary results indicate that anti-RaLV antibody behaves similarly when added to cultures of radiation-treated F1706 cells.

Spontaneous transformation of RaLV-producing Sprague Dawley (SD-1) cells was prevented by treatment with RaLV-specific antisera. Studies are in progress to demonstrate the effect of RaLV antisera on accelerated transformation by various chemical carcinogens in this cell system. (3) An antigen found in all rat tumors, whether induced by chemicals and/or viruses, could be extracted by Triton X-100 and by hypotonic buffer with little loss in CF activity; the activity banded in gels at approximately 155,000, 120;000 and 30,000 daltons. Experiments are underway to determine the effectiveness of this antigen for immunoprevention of cancer in the rat as well as in other mammalian systems.

CONTRACT REPORTS
COCARCINOGENESIS STUDIES SECTION

Dr. Lea I. Sekely

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP7-1017)

Title: Genetic and Immunologic Factors in Viral Leukemogenesis

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. John Stephenson

Objectives: Conduct studies on the relationship of infection with leukemia viruses and the response to topically applied chemical carcinogens.

Major Findings: (1) Studies of spontaneous leukemia in the AKR x RF cross. The RF strain, previously considered to have the same Fv-1^n allele as AKR, apparently has a variant allele, here called Fv-1^{nr}. This allele, in crosses with AKR, did not suppress the expression of ecotropic MuLV but also appeared to suppress expression at 6 months of age of xenotropic MuLV in the thymus. This X-MuLV suppression then appeared to be the cause of the fact that the Fv-1^{nr} allele also suppressed the occurrence of the AKR type of leukemia in these crosses. There was also a marked maternal effect on leukemogenesis in this system: either AKR mothers transmit an additional, nonchromosomal susceptibility factor, or RF mothers transmit similarly a resistance factor for the leukemia. Implantation of X-MuLV-positive 6 month AKR thymus cells into 2 month AKR and AKR/RF F_1 mice induced X-MuLV expression in AKR but not F_1 host thymus tissues. (2) Studies of MCA-induced leukemia. The Ah locus seemed in earlier experiments to be responsible for determining if MCA painting would result in skin tumors or in leukemia. A study in a segregating generation, (RF x C3H) x RF, has not been completed, and the findings confirm the hypothesis. In this cross, two basic phenotypes in response to MCA painting appeared: (a) AHH-inducible (Ah^b), skin tumor-susceptible, leukemia-resistant, and (b) AHH-noninducible (Ah^d), skin-tumor-resistant, leukemia-susceptible. Ah^b mice with high levels of endogenous MuLV expression, however, showed a markedly attenuated skin-tumor response and AH^d mice may require a particular type of endogenous MuLV in order to develop the expected leukemic response. Preliminary experiments in various types of mice give conflicting results concerning the question of whether or not MCA painting induces MuLV expression. X-MuLV expression was induced in (AKR x RF) F_1 mice, but neither X-MuLV nor eco-MuLV was induced in RF mice, although both types of mice were equally susceptible to MCA leukemogenesis.

Significance to Biomedical Research and the Program of the Institute: One of the basic facts about tumor biology is that genetic mechanisms of the host exert major control over the expression of oncogenicity. By defining the loci and markers associated with leukemogenesis, it should be possible to undertake systematic studies of the precise immunochemical mechanisms governed by individual loci, with the objective eventually of encouraging or altering immunogenetic effects to provide maximum resistance against cancer. The identification of chemical markers associated with cancer resistance and

susceptibility could also prove important in identifying cancer susceptible individuals or in early diagnosis of the disease.

Proposed Course: Emphasis will be placed on the study of viral and genetic factors in influencing chemically-induced leukemia.

Date Contract Initiated: May 13, 1965

ENERGY, DEPARTMENT OF (Y01-CP7-0503)

Title: In Vitro Interaction of Chemical Carcinogens with Primate Cells: Relationship to Expression of Endogenous Primate or Exogenous Murine Viruses

Contractor's Project Director: Dr. George C. Lavelle

Project Officer (NCI): Dr. Stephen Tronick

Objective: To obtain new information on the importance of the expression of oncornavirus genes in chemically-induced transformation events in primate cells, the contractor will: (1) establish an in vitro transformation assay system utilizing diploid fetal baboon cells, (2) determine if chemical carcinogens have a potentiating effect on transformation by oncornaviruses in primate cells and correlate such transformation with the expression of baboon endogenous virus and (3) determine the effect of chemical carcinogens on primate cells expressing xenotropic oncornavirus.

Major Findings: A strain of whole baboon embryo (WBE) cells persistently released low levels of BaEV. A second strain of fetal skin and muscle (FSM) cells were uniformly negative for virus even after prolonged cocultivation with susceptible indicator cells. However, FSM cells are permissive for infection by BaEV. This finding was confirmed for 3 strains of baboon cells (from different animals, fetal and adult) and 3 independent isolates of BaEV. Infection of FSM cells by the M7 strain of BaEV appears to be single-hit.

Karyotype analysis confirmed that FSM and WBE cells are of baboon origin. Their doubling time and plating efficiency were characterized.

Cocultivation of FSM cells with several lines of indicator cells did not result in release of endogenous virus by the supernatant RNA-dependent DNA polymerase (RDDP) assay even after multiple subcultures. However, by use of the cellular immunofluorescence assay with anti-M7 p28 serum, the contractor found that FSM cells expressed viral p28 within 2 days after treatment with bromodeoxyuridine (BUDR). RDDP assays of treated culture fluids remained negative, and did not demonstrate release of infectious virus by cocultivation. Virus particles were not found by electron microscopy. Preliminarily, it therefore appears that at least one of the viral gag genes was expressed in response to BUDR treatment, but that complete virus was not produced. Induction of viral p28 in this system had characteristics of virus induction in the

murine system, namely, requirements for BUDR incorporation and for cell proliferation.

In cocarcinogenesis experiments, the interaction of several chemical carcinogens with virus-positive and virus-negative cells were studied. FSM cells treated with BUDR, or persistently infected with M7 virus, or infected with a murine xenotropic pseudotype of Kirsten sarcoma virus, as well as untreated cells, failed to metabolize PAH - benzo(a)pyrene or 3-methylocholanthrene - to their carcinogenic intermediates. However, two of the purified intermediates, i.e. dihydrodiol and the diol-epoxide of benzo(a)pyrene have toxic and growth inhibitory effects on normal and virus-expressing baboon cells. Further characterization of these interactions are in progress.

Significance to Biomedical Research and the Program of the Institute: This project is directed at the development of primate-derived cell-virus transformation assay systems in order to provide basic data on the potentiation by endogenous and xenotropic viruses of chemical carcinogenesis in primates.

Proposed Course: Continuation of activities.

Date Contract Initiated: November 1, 1977

ENERGY, DEPARTMENT OF (Y01-CP7-0504)

Title: In Vivo Radiation-Activation of Endogenous Sarcoma Virus Genome

Contractor's Project Director: Dr. Emerson Chan

Project Officer (NCI): Dr. John Dahlberg

Objectives: To ascertain the cofactorial role of oncornaviruses in the induction of malignancies in mice by ionizing radiation, the contractor will: (1) characterize the FBJ and FBR murine viruses and determine the identity and nature of sarcogene sequences in normal mice tissues and radiation-induced osteosarcomas and (2) determine whether activation of endogenous sarcogenes/virogenes by radiation is an underlying mechanism of the neoplastic process.

Major Findings: The biochemical characterization of the murine osteosarcoma viruses, FBJ and FBR, were continued. Collaborative study showed that FBJ virus was genomically different from Moloney sarcoma virus. Moloney "src" sequences were absent in FBJ viruses and the "leuk" and "com" sequences were poorly related.

Analysis of viral proteins by SDS gel electrophoresis revealed FBJ and FBR had small peptides of unique size-classes. Instead of the usual p10, p12, and p15 of murine type C viruses, they have p9 and p11. Since low molecular weight polypeptides usually carry type-specific antigenic determinants, these distinctive differences were of interest in the development of specific radioimmunoassays for these viruses.

Further testing in tissue culture confirmed that the best cells for growing up FBJ and FBR were the BALB/c and Sc-1 fibroblastic cell lines, respectively. These infected cell lines consistently produce high titers of transforming viruses (10^5FFU/ml) which are potently osteosarcomagenic. The associated non-transforming components were present in excess by only one log. Such low transforming:non-transforming ratios made the proposed attempts to synthesize sarcoma-specific ^{3}H-cDNA probes, a feasible one. The contractor is continuing his efforts to isolate type C transforming and tumorigenic viruses, like FBJ and FBR, from ^{90}Sr-induced osteosarcomas of CF#1 and X/Gf mice.

To date results indicate that most, if not all, extracts made from ^{90}Sr-induced osteosarcomas contain viruses detectable by infectivity assays monitored by reverse transcriptase and XC plaque tests. Similarly, the majority, if not all, tumors placed in tissue culture or co-culture with susceptible host cells (BALB/c, Sc-1), also produce infectious viruses detectable by the same tests. These viruses are being evaluated for their transforming and tumorigenic potentials.

Significance to Biomedical Research and the Program of the Institute: This project will further identify and characterize endogenous murine viruses and elucidate the role of oncornaviruses in the radiation-induced oncogenic process. These studies will extend the knowledge by which radiation induces neoplasia in animals and man by determining the specific role of putative endogenous type C oncornaviruses in radionuclide-induced bone cancer in mice.

Proposed Course: Continuation of activities.

Date Contract Initiated: November 1, 1976

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP4-3240)

Title: Viral-Chemical Carcinogenesis Studies

Contractor's Project Director: Dr. Paul J. Price

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To develop, evaluate, standardize and apply *in vitro* systems for studying viral-chemical cocarcinogenesis using known and suspected carcinogens in the environment. Utilize and complement *in vitro* systems to elucidate viral and cellular components in cell transformation, including the role of endogenous viruses. Utilize cell systems to study potential anti-cancer products, including viral vaccines, viral inhibitors, etc., to elucidate the role of type C viruses in transformation. Utilize and adapt subhuman transformation and viral induction procedures to studies of human tumor and transformed cell lines for assaying suspected chemical carcinogens. Study the effect of viral vaccines, viral inhibitors, interferon and/or genetic regulation of endogenous virus on naturally occurring and chemically induced tumors.

Major Findings: The F1706 Fischer rat embryo transformation assay system which was developed in this laboratory has been shown to be a sensitive indicator of chemicals carcinogenic for rats. This system was used to determine the potential oncogenicity of benzedrine, dexedrine and ritalin. In two separate experiments benzedrine was found to be a weak transforming agent while ritalin was found not to be a transforming agent. Dexedrine did not transform the cells in one experiment but was a weak transforming agent in the second. These experiments are being repeated using a wider range of dosages. The F1706 system was optimized with respect to planting concentration prior to and after chemical treatment. Initial seeding of 500 cells per ml 24 hours prior to chemical treatment, followed by a post-chemical treatment seeding of 500 or 5000 cells per ml, gave the optimal results. Attempts to develop a carcinoma-equivalent transformation assay system were initiated using epithelial cultures isolated from adult Fischer rat spleens and grown on a rat tail collagen substrate.

Earlier observation that 4 neutralizing units of goat IgG specific for the normally unexpressed endogenous virus (RaLV) inhibits chemical transformation of F1706 if added to the cells 72 or 48 hours prior to 4NQO treatment and enhances transformation if added at the same time or after 4NQO treatment was repeated and confirmed. Equally toxic dilutions of goat antibody to AT124, RLV or GLV did not protect the cells from transformation. In a preliminary experiment, goat anti-RaLV antibody (4 neutralizing units) also protected F1706 cells from transformation induced by 200r from a cesium source. GLV antibody was ineffective. Only the phenotypically trensformed cells grew in semisolid agar and were tumorigenic in newborn Fischer rats. Experiments were also initiated to demonstrate the role of the endogenous oncornavirus in spontaneous and chemically induced transformation of mouse cells. Low passage BALB/c and Swiss mouse embryo cells were propagated in the presence or absence of specific antiviral antibodies and screened for spontaneous transformation. Using the C3H 10 T 1/2 clone 8 cells, it was found that the induction of endogenous virus by IUDR was first observable around subculture 17. Attempts to induce viral expression at subculture levels 5-10 were all negative. At subculture levels above 23, the 10 T 1/2 cells spontaneously expressed both viral activity and transformation. Inhibition of chemical transformation in the 10 T 1/2 cells by goat anti RadLV (2 neutralizing units) antibody was unsuccessful.

Significance to Biomedical Research and the Program of the Institute: This contract has established the feasibility of using tissue culture systems for screening environmental compounds for carcinogenic potential in a fraction of the time and cost of screening in animals.

The development of materials and methods for active or passive immunization against carcinogenesis have important implications for cancer prevention and control.

Proposed Course: This contract terminated June 1, 1978

Date Contract Initiated: February 1, 1970

MICROBIOLOGICAL ASSOCIATES, INC. (NOI-CP5-3519)

Title: Isolation and Characterization of Type C RNA Tumor Viruses and Diagnostic Testing and Service Functions

Contractor's Project Director: Dr. Johng S. Rhim

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To define the events, with special emphasis on the role of endogenous type C RNA viral genomes, associated with activation of oncornaviruses in nonproducer cell cultures by chemicals, DNA viruses, or spontaneously; and to characterize the endogenous viral isolates.

Major Findings: Attempts were made to isolate viruses from fresh human cancer material by cocultivation with various cells, and to activate or rescue sarcoma virus from nonproducer human osteosarcoma cells that contained KiMSV genome (NP-KHOS). Non-tumorigenic revertants of NP-KHOS cells supported leukemia virus growth and showed enhanced sensitivity to MSV superinfection but showed no evidence of the KiMSV genome by all techniques used to date. The revertant cells were readily transformed by 3MC and DMBA producing tumors when injected into NIH nude mice and thus may be useful in screening for possible human chemical carcinogens.

A type C virus was activated from cultured normal rat liver cells using IUDR as determined by RNA-dependent DNA polymerase but rat leukemia p30 antigen was not detected in the IUDR treated cells. Further studies are in progress. A guinea pig herpesvirus (GPHV) which was isolated and shown to have a narrow host range has now been shown to transform rat embryo cells. However, the transformed cells do not produce infections or viral antigens and the virus has not been rescued by cocultivation with guinea pig cells. The transformed cells grow in aggregate form, form colonies in soft agar and produce tumors when transplanted subcutaneously into newborn rats. Herpesvirus complement-fixing antigen was detected in the rat tumors but infectious virus was not recovered. The GPHV transformed rat embryo cells produced a CF antigen shared in common with other transformed rat cells and probably represents the common rat tumor antigen. The common rat tumor antigen, which was partially characterized, can be extracted by Triton X-100 and by hypotonic buffer with little loss in CF activity, was not detectable by the staphylococcal protein A adsorption test and immunofluorescence and yielded three bands of approximately 155,000, 120,000 and 30,000 daltons. Growth of cells in the aggregate form was evaluated and shown to have the potential for a fast and accurate means of evaluating *in vitro* transformation.

Significance to Biomedical Research and the Program of the Institute: The isolation of human type C RNA viruses, and the definition of events associated with expression of virus or viral products would be the major handle for controlling cancer, either through the use of preventive vaccines or by interruption of the processes leading to virus expression and neoplasia.

Proposed Course: Continue studies outlined in above Objectives Section

Date Contract Initiated: November 23, 1974

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP6-1043)

Title: In Vitro Malignant Transformation of Human and Subhuman Primate Cells by Interaction between Viruses and Chemicals

Contractor's Project Director: Dr. Aaron Freeman

Project Officer (NCI): Dr. Robert Bassin

Objectives: To determine whether chemical carcinogens enhance or potentiate viral oncogenic transformation of primate cells in vitro and to elucidate the mechanisms of action, the contractor will treat human cell cultures persistently infected with oncogenic virus with chemical carcinogens and observe for and confirm malignant transformation events.

Major Findings: A cocultivation system that consists of islands of skin epithelial cells surrounded by type C virus-infected fibroblasts has been developed.

The principal investigator and coworkers have initiated a number of experiments in which baboon leukemia virus-infected human skin fibroblasts have been cocultivated with human skin epithelial cells and exposed to chemical carcinogens. Progeny of the treated cultures have been subpassaged and periodically frozen for up to 50 population doublings. A number of cultures have been characterized in terms of certain characteristics, e.g., morphology, karyotype, growth in soft agar.

A number of suggestive but undefined observations have been made. Certain cultures contain foci of altered cells which have not yet been identified. Some cultures have heteroploid cell populations which have not yet been characterized. Some cultures produce multiple colonies in soft agar, but no tumor data in nude mice is yet available.

Significance to Biomedical Research and the Program of the Institute: These in vitro studies may result in the establishment of relevant, rapid and inexpensive test systems for identifying environmental carcinogens with potential for causing human cancer.

Proposed Course: Continuation of activities

Date Contract Initiated: September 29, 1976

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP6-1045)

Title: Development of Mammalian Cell Lines Known to Contain Endogenous Oncogenic Virus Sequences which can be Utilized in Testing Mutagenic and Carcinogenic Effects of Environmental Factors

Contractor's Project Director: Dr. Paul Price

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To develop cell lines (or strains) from species known to contain integrated oncornavirus sequences and to use such *in vitro* systems in cocarcinogenesis assay, the contractor will: (1) develop *in vitro* cell lines known to contain integrated oncornavirus sequences; (2) determine transformation frequencies when exposed (and not exposed) to chemical or physical carcinogens; (3) confirm malignant transformation of morphologically altered cells; and (4) correlate mutagenic and carcinogenic activities with each other and determine the mechanisms of action and target sites for the carcinogens tested.

Major Findings: Base line studies on the human xeroderma pigmentosum (XP), human splenic "B" lymphocytes, and chimp lung cell lines were initiated and completed. Each cell line was characterized as to AHH inducibility, karyology, growth in agar, morphology, absence of microbial contaminants, life span, carcinogen toxicity, and tumorigenicity in the nude mouse. All lines were treated with LD-30 (dose reducing plating efficiency by 30%) and MNTD (maximum non-toxic dose) of B(a)P and MNNG as outlined in the proposal. All cell lines became senescent with the exception of XP cells treated for 1 week with B(a)P. This cell line remained sensitive to U.V., grew rapidly, formed colonies in agar, grew in low serum concentrations, and was phenotypically altered. Chromosome analysis showed that the cell line was still human female but heteroploid. Large pools have been frozen to look for "src" expression; XP cells were also treated with chemical after arginine deprivation and in the presence of carcinogen metabolizing rat hepatocytes. Splenic "B" lymphocytes were grown on rat-tail collagen (these cells were different morphologically from the same cells grown on plastic) and were treated with carcinogen. Chimp lung, human XP, and human splenic "B" lymphocytes were nontumorigenic in nude mice.

It was confirmed that mutations can be induced in human cells using a human cell activating system. Both oua^t and $6\text{-}TG^r$ mutations have been induced by the procarcinogen MCA.

Significance to Biomedical Research and the Program of the Institute: Primate cell lines might yield an assay system more relevant to the human situation as well as providing important and vital information on the basic biology of malignant transformation and mutation.

Proposed Course: This contract terminated March 28, 1978

Date Contract Initiated: September 29, 1976

PENNSYLVANIA STATE UNIVERSITY (NO1-CP6-1063)

Title: *In Vitro* Malignant Transformation of Human and Subhuman Primate Cells by Interaction Between Viruses and Chemicals

Contractor's Project Director: Dr. Fred Rapp

Project Officer (NCI): Dr. Masakazu Hatanaka

Objectives: To determine whether chemical carcinogens enhance or potentiate viral oncogenic transformation of primate cells *in vitro* and to elucidate the mechanisms of action, the contractor will treat human or nonhuman primate cell cultures persistently infected with virus with chemical carcinogens and observe for and confirm malignant transformation events.

Major Findings: To examine cocarcinogenesis in a human transformation assay, an assay for transformation of the human osteosarcoma cell line, HOS, was being established. Morphologically transformed clones of HOS cells have been derived after exposure of cells to 8 plaque-forming units (pfu)/cell of HSV. These clones possess HSV-specific antigens and were superinfectable by virus although to a lesser extent than the parent HOS line. After transformation, the fibrinolytic activity of these lines was greatly increased. High levels of fibrinolytic activity was also demonstrated in other herpesvirus-transformed lines. Presently this assay is being quantitated for use in carcinogen studies.

Continued studies with AD12 transformation of rat embryo fibroblasts have shown that infection of cells 4-6 hours after treatment with 0.2mM N-methyl-N-nitrosourea (NMU) resulted in threefold enhancement of normal transformation levels. Preliminary studies to examine macromolecular synthesis after 0.2mM NMU treatment show that there was a small burst of thymidine incorporation during the time corresponding to transformation enhancing potential. This thymidine incorporation might have represented repair synthesis of DNA.

Significance to Biomedical Research and the Program of the Institute: Several DNA viruses, notably the papovaviruses, herpesviruses and adenoviruses have been shown to possess transforming or oncogenic potential in laboratory animals. This contractor will attempt to determine whether a synergistic effect exists between carcinogens and DNA viruses in the process of malignant transformation. The effort also may provide insights into whether DNA tumor viruses mediate cellular transformation by activating endogenous host oncogenic sequences. These studies may provide *in vitro* test systems vitally needed for rapid screening of potential carcinogens.

Proposed Course: Continuation of activities

Date Contract Initiated: September 29, 1976

SAINT LOUIS UNIVERSITY (NO1-CP6-1062)

Title: Influence of Interactions Between Environmental Factors (and Viral Sarcogenes in Malignant Transformation of Murine Fibroblasts)

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Stephen Tronick

Objectives: To further elucidate the cofactorial relationships between carcinogens (chemical, physical, and/or biological) and endogenous type C RNA viruses as these relate to activation of endogenous "sarcogene" expression and malignant transformation of cells in vitro, the contractor will: (1) determine the correlation between sarcogene activation by carcinogens and malignant transformation of cells, and (2) determine the nature and mechanisms of the interaction of the carcinogenic cofactor with the cell which leads to activation of the cellular/viral genetic information.

Major Findings: The contractor prepared frozen banks of cells (ranging from 0 to 30 population doublings) from individual embryos and embryo pools of NIH Swiss and BALB/c mice. Cells were treated with 3-methylcholanthrene (MCA), with and without coinfection with AKR virus, and cell cultures passaged accordingly to vertical-horizontal protocols. Cells were viably frozen at every fifth population doubling. Cells at 30 population doublings have been examined for transformed cell-related characteristics, including morphology, plating efficiency in liquid, cloning in soft agar, aryl hydrocarbon hydroxylase activity, karyology, and tumorigenicity in nude mice. Various cell cultures exhibited an array of these transformation-related characteristics, ranging from untransformed to highly transformed. Cultures of D30 were tested for their tumorigenicity in nude mice and it was found that increases with dose of MCA and the passage level. The parallel control cultures were not tumorigenic. The same culture was available at two passage levels, one tumorigenic and one nontumorigenic. None of the conventional markers of transformation correlated completely with chemical dose and tumorigenicity. Cell morphology gave fairly good correlation, but growth in soft agar gave poor correlation. Heteroploidy was a general occurrence in mouse cells and was not related to tumorigenicity. Src-specific RNA in 24 MCA-transformed cell cultures and MCA-induced tumors was not detected. Thus, there was no evidence that MCA-transformed cells express the M-MSV sarcogene. Tumorigenic cell cultures at different passage levels were being analyzed to test for transient expression of src-RNA. Using the ^{3}H-cDNA(sarc) probe, the contractor analyzed several clonal lines of nonproducer MSV-transformed NIH Swiss cells that displayed different "degrees" of transformation, as measured by concanavalin A agglutinability, morphology, and synthesis of MLV-specific RNA. The quantity of src-RNA in these cells correlated strongly with the degree of transformation.

In attempts to prepare an immunological sarcoprotein probe, antisera in rabbits was prepared against MSV transformed (S+L-) NIH Swiss cells. After absorption with normal NIH/3T3 cells, this antiserum immunoprecipitated two strong polypeptides from MSV-transformed cells but not from normal cells.

Significance to Biomedical Research and the Program of the Institute: The synergistic effects of chemical or physical carcinogens and viruses, in inducing cell transformation in vitro and tumor production in vivo have been demonstrated. The mechanism(s) of action and the relative importance of the interaction between these putative cocarcinogens is not well understood. Because it is not feasible to eliminate most carcinogens from the environment, only a thorough understanding of the basic biology of malignant transformation, including the mechanisms by which viruses or viral genetic information and other carcinogens interact, is likely to indicate pratical approaches to prevention and control of many forms of cancer.

Proposed Course: This contract will terminate September 28, 1978

Date Contract Initiated: September 29, 1976

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP6-1041)

Title: Influence of Interactions Between Environmental Factors (and Viral Sarcogenes in Malignant Transformation of Rat Cells)

Contractor's Project Director: Dr. Suraiya Rasheed

Project Officer (NCI): Dr. Edward M. Scolnick

Objectives: To further elucidate the cofactorial relationship between carcinogens (chemical; physical, and/or biological) and endogenous type C RNA viruses as these relate to activation of endogenous "sarcogene" expression and malignant transformation of cells in vitro the contractor will: (1) determine the correlation between sarcogene activation by carcinogens and malignant transformation of cells, and (2) determine the nature and mechanisms of the interaction of the carcinogenic cofactor with the cell which leads to activation of the cellular/viral genetic information.

Major Findings: The principal investigator further characterized the Sprague-Dawley (SD-1) endogenous RaLV releasing rat cell system. A large number of virus producer and nonproducer fibroblast SD-1 clones have been tested for transformation by chemical carcinogens. Only those cells transformed by chemicals spontaneously produced endogenous RaLV or were exogenously infected with this virus. These findings have been confirmed on several SD-1 single cell clones as well as mass SD-1 cultures using a direct acting chemical carcinogen, N-methyl-N'-nitro-N-nitrosoquanidine (MNNG). SD cells free of detectable RaLV production would not transform when treated with the carcinogen.

Both in the cloned and uncloned RaLV-producing SD-1 cells, spontaneous transformation can be prevented by treatment with RaLV specific antisera. Further attempts will now be made to prevent, with RaLV antisera, the acceleration of transformation by various chemical carcinogens.

A type C virus from a wild rat was isolated and characterized. By interference and virus neutralization tests the virus was related to other RaLV isolates from various laboratory rat strains. However, it differed from SD or Fischer RaLV (F-RaLV) in its lesser infectivity for rat cells and its inability to accelerate transformation of infected rat embryo cells. The wild rat or SD- or F-RaLV's have not produced any disease in, or been infective for, newborn rats.

Preliminary data suggest that a stable fibroblast transforming virus was isolated from two different chemically transformed rat cells infected with SD-RaLV. The biochemical and biological studies are now in progress to further elucidate the molecular interaction of cellular and/or viral genes and the role

of src sequences in the pathogenesis of spontaneous and chemical tumors in rats.

Significance to Biomedical Research and the Program of the Institute: The synergistic effects of chemical or physical carcinogens and viruses in inducing cell transformation in vitro and tumor production in vivo have been demonstrated. The mechanism(s) of action and the relative importance of the interaction between these putative cocarcinogens is not well understood. Because it is not feasible to eliminate most carcinogens from the environment, only a thorough understanding of the basic biology of malignant transformation, including the mechanisms by which viruses or viral genetic information and other carcinogens interact is likely to indicate credible approaches to prevention and control of many forms of cancer.

Proposed Course: Continuation of activities

Date Contract Initiated: September 29, 1976

TEXAS, UNIVERSITY OF (NO1-CP6-1042)

Title: In Vitro Transformation of Mammalian Cells Resulting from the Intracellular Interaction of a Non-oncogenic Virus and Chemicals

Contractor's Project Director: Dr. Thomas Albrecht

Project Officer (NCI): Dr. Robert Goldberg

Objectives: To explore the possibility that interactions between chemical carcinogens and cells persistently infected with a non-oncogenic virus may result in malignant transformation the contractor will: (1) develop in vitro cell culture systems persistently infected with normally non-oncogenic viruses; (2) subject such cultures to insult by carcinogenic chemicals; (3) monitor treated and control cell cultures for morphological transformation, activated expression of endogenous oncornavirus information and altered expression of the non-oncogenic virus; and (4) confirm the malignant potential of transformed cells by in vivo inoculation of appropriate host.

Major Findings: Human cell lines persistently infected with human cytomegalovirus have been established using human embryonic skin muscle or lung cell cultures and either relatively recent virus isolates or laboratory strains of plaque type 3. Initially cytopathology was present in these cultures as evidenced by swollen cells containing both nuclear and cytoplasmic inclusions. With additional subcultures, the virus cytopathology became undetectable and the cells were submitted to further study. A very few percent of these cells contained internal CMV-specific antigens, yet thus far the contractor has been unable to isolate infectious virus. Cytomegalovirus-specific antigens were demonstrated in a greater percentage of the cells following IUDR induction.

Exposure of these persistently infected cells to chemical carcinogens has demonstrated two contrasting phenomena. Some of the chemicals induced cytopathology and productive replication of cytomegalovirus. These cultures were destroyed as a result of the subsequent lytic infection. Other chemicals caused further morphological alteration of the cells and occasionally limited cytopathology. These latter cell lines were characterized.

Significance to Biomedical Research and the Program of the Institute: The synergistic effects of chemicals and oncogenic viruses in malignant transformation of cells has been demonstrated. Similarly, several normally non-oncogenic DNA viruses have been shown to be oncogenic after treatment by chemical or physical factors. This project will further the understanding of the possible importance of the interaction of chemical carcinogens and normally non-oncogenic viruses as related to malignant transformation.

Proposed Course: Continuation of activities.

Date Contract Initiated: September 27, 1976

SUMMARY REPORT

C. 1. b. (4) DNA VIRUS STUDIES SECTION

October 1, 1977 through September 30, 1978

The DNA Virus Studies Section is concerned with research to explore the possible etiological relationship of DNA viruses to human malignancy. The studies are divided into two major categories: (1) studies of direct DNA virus associations with human neoplastic disease; and (2) studies in animal tumor model systems. The first category includes DNA viruses such as the Epstein-Barr viruses (EBV), the herpes simplex viruses (HSV), the papovaviruses, and the adenoviruses. The second category includes studies of herpesviruses in nonhuman primates and in lower vertebrates.

DNA Viruses Associated with Human Neoplastic Diseases.
EBV Studies: Further studies of the state of the EBV genome in long established Burkitt lymphoma (BL) lines and in recently established African BL lines showed that integrated and free circular DNA sequences occurred similarly in both lines. This indicates that the characterization of EBV DNA in long established lines is not a secondary artifact of tissue culture methodology. A number of lymphoblastoid cell lines of non-neoplastic origin contained small EBV DNA circles in contrast to the large circles seen in BL cells. The small circles are characteristic of cells infected with the B95-8 virus strain and the inference may be made that circle size reflects a viral strain difference rather than a disease related difference. In cell lines that contain one to four EBV genome equivalents per cell, all detectable copies were present as integrated viral sequences and there were no free covalently closed circles. In contrast, EBV immortalized cord cell lines had virtually all of the viral DNA present as free covalently closed circles. However, if the cord cells were treated with lipopolysaccharide at the time of EBV exposure, most or all of the viral DNA was present in the integrated form.

The amount of Epstein-Barr virus-directed nuclear antigen (EBNA) was found to be directly proportional to the number of EBV genome copies measured in a variety of EBV-carrying cells and hybrids as well as in a series of completely nonpermissive and noninducible heterologous hybrids between human EBV-carrying cells and mouse fibroblast or carcinoma cells. This indicates that EBNA induction is a relatively autonomous function of the viral genome and is not subject to a cellular regulating mechanism as are all other known EBV products.

Studies on correlating persistence of EBV genomes with a specific human chromosome were conducted on a large series of hybrids derived from the fusion of nasopharyngeal carcinoma (NPC) biopsy cells and mouse fibroblasts. Results showed that EBV DNA and EBNA can be maintained after most of the human chromosomes are lost and, conversely, can be lost at a time when a considerable number of human chromosomes are still present.

In studies on the clinical course of Burkitt lymphoma (BL), preliminary data suggest a relationship between antibody-dependent lymphocyte cytotoxicity (ADLC) titers and a favorable clinical course. Long term BL survivors have significantly higher titers than patients who died with recurrences and metastases. In 51 undifferentiated carcinomas of the nasopharynx, all were found to contain EBV DNA with multiple viral genome copies per cell. However 6 of 7 lymphomas localized in the nasopharynx were EBV DNA negative; the positive one corresponded histologically to BL. Head and neck carcinomas outside the nasopharynx were all EBV DNA negative. These results confirm the regular and unique association of EBV DNA with undifferentiated carcinomas of the nasopharynx.

HSV Studies: A new serological technique, the microtiter solid phase radioimmunometric assay, was developed to detect type specific antibody in human sera to HSV-1 and HSV-2 antigenic determinants without the necessity of prior absorption of the test sera. This method may prove invaluable for the conduct of sero-epidemological studies of uterine cervical carcinoma in an available prospective-retrospective cohort. An early antigen, AG-4, extracted from HSV-2 infected HEp-2 cells, has been purified and used in a preliminary serological survey of women with cervical cancer. AG-4 appeared to be reactive only with sera from cervical cancer patients, thus confirming data previously published by another contractor's laboratory. Several assays were used to detect cell-mediated immunity in cervical cancer patients in order to obtain further evidence of an association between HSV-2 and cervical neoplasia. No evidence of greater reactivity in women with HSV-2 infection as compared to controls was obtained and no marked cytotoxic differences were shown between women with cervical anaplasia, as compared with controls, when target HSV-2-infected cells were used.

The basic concept of the usefulness of cell line specific DNA probes for HSV-2 was tested in a series of additive hybridization experiments. The results of these experiments clearly showed that all of the cell lines tested, both morphological and biochemical transformants, shared a viral DNA fragment approximately 2 x 10^6 daltons in size. Studies on a series of revertants of biochemical transformants indicated that expression of viral TK is subject to positive control by a viral gene not adjacent to the structural gene for TK. The TK region of the HSV genome becomes very stably associated with the cellular DNA and even in "revertant" clones there is evidence for the continued presence of the HSV TK gene. These conclusions are based on a study of the TK activities found in biochemical transformants, their "revertants" and morphological transformants. Evidence obtained to date suggests that all HSV-transformed cells have a common DNA sequence and the viral TK gene is part of that nucleic acid sequence.

A quantitative transformation assay was developed to determine whether specific growth factors, hormones, or combinations of these agents potentiate HSV transformation. Cyclic AMP caused a slight increase in transformation, theophylline caused a marked decrease, and diethylstilbestrol appeared to inhibit transformation. Characterization studies of the HHCV herpesvirus which has biological and biochemical characteristics intermediate to cytomegalovirus (CMV) and HSV have continued. In rabbit kidney cells, growth is similar to that of HSV-2; in human diploid fibroblasts, virus

growth is like CMV. Both primary human kidney and endometrial cells are transformed by HHCV. The HHCV DNA buoyant density is slightly higher than the buoyant density of HSV DNA and is far removed from the buoyant density of CMV. The restriction enzyme cleavage pattern is distinctly different from HSV and CMV. RNA-DNA hybridization studies show less than 10% homology with HSV-1, about 30% with HSV-2, and no homology with laboratory adapted strains of CMV.

Papovavirus and Adenovirus Studies: Human vascular endothelial cells (HEC) transformed by the human papovavirus, JC, were compared to primary human umbilical vein endothelial cells with regard to their production of specific extracellular materials. The endothelial cells, whether or not transformed, release fibronectin and another prominent material labeled with ^{3}H-proline or lysine into the cell culture medium. They also release one or more species of basement membrane collagens, but no other collagens, into the medium if they are transformed. This basement membrane collagen is probably larger than interstitial procollagens and probably contains individual chains in disulfide linkage. Its behavior is consistent with a triple collagen helix containing a few pepsin or trypsin sensitive sites and some nonhelical regions.

The Southern gel electrophoretic-hybridization procedure was used to demonstrate the presence of both free and integrated BK papovaviral DNA sequences in T-antigen negative transformed human fetal brain cells. Acute infection of primary human fetal brain cells with human papovavirus BK has been shown to cause extensive cellular vascularization and destruction. A small fraction of surviving cells can be recovered by frequent medium changes. These survivors, BK-HFB cells, have some properties of transformed cells (rapid growth rate, increased saturation density, colony formation in soft agar) and are persistently infected with BKV. Unlike SV40 in human cells or polyoma virus in mouse cells, only a small percentage of the cells are T-antigen positive, corresponding roughly to the number of virus-producing cells in culture.

To investigate whether ubiquitous oncogenic human DNA viruses cause human cancer, tumor DNAs and RNAs were assayed for viral "transforming genes," using in vitro labeled viral DNA and transforming DNA restriction fragments as probes for molecular hybridization. Over 2500 human tumors were collected, and nucleic acids extracted from 800. The 29 human adenovirus (Ad) serotypes formed five distinct DNA homology groups: group A (Ad12, 18, 31), group B (Ad3, 7, 11, 14, 16, 21), group C (Ad1, 2, 5, 6), group D (Ad8-10, 13, 15, 19, 20, 22-24, 26-30), and group E (Ad4). Ads within each group are 60-100% homologous but are <20% homologous to other Ads. In vitro labeled transforming fragments of group A Ads (Ad12 strain Huie EcoRI-C, left 16% of genome) and group C Ads (Ad HindIII-C, left 7.5% of genome were prepared. With these probes, no Ad DNA sequences were detected in 88 tumors using Ad12 transforming EcoRI-C as probe, and 113 tumors using Ad5 HindIII-C. These probes could detect one copy per tumor cell of about 1-3%, or 0.5%, respectively, of the Ad genome. Therefore these results virtually exclude Groups A and C Ads as etiological agents for these tumors. One hundred fifty-six tumors were assayed for human papilloma virus type 1 (HPV-1 plantar warts), and 145 tumors for HVP-2 (hand warts). No viral sequences

were found. The HVP-1 and HVP-2 DNAs are only 5-8% homologous, consistent with other evidence for several distinct HVPs. Fifty-three normal tissues, 166 tumors, and seven malignant human cell lines were assayed for BK virus sequences. No viral sequences were detected. The sensitivity is one copy per cell of 5-10% or 0.1 copy of 100% of the BKV or HVP genome. Thus, no evidence has yet been obtained that human Ads, HVPs, or BK virus cause tumors representing 40-50% of cancers in the U.S.A. More work needs to be done, especially with group B, D, and E Ads, with other types of HPVs, and other tumor categories.

Herpesviruses in Nonhuman Primates and Lower Vertebrates.
Herpesvirus Saimiri Studies: A newly isolated highly oncogenic variant strain of Herpesvirus saimiri (HVS) was shown to produce malignant lymphoma consistently with a short incubation time in owl monkeys and common marmosets. Further characterization studies of HVS indicated that attentuation of the oncogenic properties occurred following passage in Vero cells, human cell cultures, and dog fetal lung cultures. Lymphocyte transformation by HVS was stimulated in the African green monkey although lymphoma was not induced. New Zealand white rabbits were susceptible to malignant lymphoma and leukemia following injection with tissue culture preparations and purified HVS. Purified HVS linear DNA molecules induced malignant lymphoma in Saguinus oedipus. With further delineation of the properties of HVS, this virus may become useful as an EBV model for development of technologies to assess immunity; studies on course of disease; acquisition of information of events at the molecular level in malignant transformation; and development of disease prevention measures.

Marek's Disease Herpes Virus Studies: In studies on the role of Marek's disease herpes virus (MDHV) in Marek's disease of chickens, transformed nonproducer MKT cells, established from a Marek's disease kidney tumor, contained 25 genome copies per cell, mostly as circular plasmid DNA. IUDR treatment of the cells increased transcription of the virus genomes. Coinfection of chickens with MDHV and a high dosage of avian leukosis virus (ALV) resultedin higher mortality of the infected birds as compared with birds infected with either virus. However, MDHV tumor induction was suppressed by the coinfection. With the development of an improved tissue culture medium for the propagation of larger quantities of MDHV, studies on the molecular virology of the interaction between MDHV and ALV are proceeding at a rapid pace.

HSV Studies: In studies of HSV-2 as a potential agent of cervical carcinoma in Cebus monkeys, 19 of 49 vaginally infected females have developed persistent abnormal cytology. Nine have never demonstrated cytologically abnormal smears. Cytological changes identified to date are of the mild (atypia or Class II) or moderate (dysplasia or Class III) nature. Histologically, dysplasia has been identified in necropsy specimens from one virus-infected animal and in a biopsy taken from a persistently abnormal virus recipient. The preliminary findings in a group of hormone-treated Cebus females suggest that diethylstilbestrol is quite active in producing cytological abnormalities in the vaginal epithelium and that estrogens with progesterone may be reacting as cofactors with HSV-2 to produce more severe and persistent cytological changes and thus, perhaps, reduce the time period for development of in situ carcinoma.

Herpesvirus Papio Studies: The DNA of an EBV-like virus, Herpesvirus papio (HVP) found in baboon lymphoblastoid cells, was present both free and integrated in the cells of five producer lines. The nonproducer lines appeared to contain only integrated HVP DNA. Both virus producer and nonproducer HVP DNA containing cell lines contained an EBNA-like antigen now designated HUPNA. Human anti-EBNA sera stained HUPNA, but no anti-HVP baboon sera stained EBNA using the acid-fixed nuclear binding technique. Because of the relationships between this baboon virus and human EBV, HVP may represent a better model for EBV than HVS in nonhuman primate systems.

BAYLOR COLLEGE OF MEDICINE (NO1-CP5-3256)

Title: Studies on Viruses Related to Cancer

Contractor's Project Director: Dr. Joseph L. Melnick

Project Officer (NCI): Dr. Berge Hampar

Objectives: To determine the relationship of viruses to selected neoplasias and their significance in the neoplastic process.

Major Findings: The use of the HSV-specific antipolypeptide sera to detect HSV gene expression in cancer cells has continued. Further studies have verified the expression of VP134 and VP143 (early nonstructural polypeptides of HSV-1 and HSV-2, respectively) with HSV-1- and HSV-2-transformed hamster cells and in human carcinoma cell lines. VP175 was detected in certain HSV-1 but not HSV-2 transformed cell lines. Antisera to the major envelope glycoprotein of HSV-1, VP123, and of HSV-2, VP119, reacted type specifically with the HSV transformed cells. Antiserum to a component of the VP123 fraction also reacted with HSV-1 transformed cells. The AG-4-like antigen from HSV-2-infected cells has been purified and appears reactive in quantitative complement fixation tests only with sera from women with cervical cancer. The purification of the DNA polymerase induced by HSV-1 and HSV-2 has enabled the elucidation of methods to detect the viral enzyme in transformed cells. Monospecific antisera to specific HSV induced DNA-binding proteins have been prepared and used to detect virus-specific antigens in transformed cells and cultured human tumor cells.

The 56S DNA of HSV-1 was cleaved with endo R. Hind III and 11 of the 14 fragments generated were used individually or in combination with one or two more fragments to transfect hamster embryo fibroblasts. Several cultures of these transfected cells have been cloned in soft agar and three of the cloned isolates were found to be positive for HSV-specific antigens. These transformed cells are being studied for (i) oncogenicity in newborn hamsters and (ii) presence of the viral-specific DNA sequences (fragments) originally used to transfect these cultures.

Rabbit antisera prepared against whole virus (HSV-1) and against two early nonstructural polypeptides (VP143 and VP175) were found to react with HSV-infected cells and with HSV-transformed cells by the immunoperoxidase technique. In addition, HSV-1 specific antigens and VP175 were readily localized by the immunoenzyme method at the ultrastructural level.

A microtiter solid-phase radioimmunoassay was developed to detect type-specific antibody present in human sera to HSV-1 and HSV-2 antigenic determinants. This test measures type-specific antibody without prior adsorption of the test serum. The test has also been adapted to identify type specificity of human isolates.

Significance to Biomedical Research and the Program of the Institute: Serological and sero-epidemiological data obtained by controlled studies on

patients with squamous cell carcinomas suggest that genital and oral strains of HSV may be factors in the genesis of these malignancies. Intensive study of the biochemical, genetic, and immunological aspects of the herpesviruses associated with these neoplasms provides information contributing to a determination of the role of these herpesviruses in the development of squamous cell carcinomas in humans.

Proposed Course: To investigate the possibility of casual relationships of HSV-1 and HSV-2 to human carçinomas, the following objective is being emphasized by the contractor: to search for virus-specific antibodies and antigens in human tissues using purified specific virion and nonvirion antigens of HSV.

Date Contract Initiated: June 27, 1963

BAYLOR COLLEGE OF MEDICINE (NO1-CP7-1058)

Title: Integration of Herpes Simplex Virus Thymidine Kinase Gene in Human Cell Genome In Vitro

Contractor's Project Director: Dr. Saul Kit

Project Officer (NCI): Dr. George Vande Woude

Objectives: To determine whether (HSV)-specific thymidine kinase (TK) is integrated on a specific chromosome and chromosome site or is episomal.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: Examination of the interactions between genomes of eukaryotic cells and oncogenic viruses may identify the basic reactions of cell regulation which are deranged by the virus and result in transformation to malignancy or which control expression of viral genetic information.

Proposed Course: This project will continue without change.

Date Contract Initiated: September 15, 1977

CALIFORNIA, UNIVERSITY OF, IRVINE (NO1-CP5-3560)

Title: Relationship of Herpes Simplex Virus Type 2 to Human Urogenital Cancer

Contractor's Project Director: Dr. David T. Kingsbury

Project Officer (NCI): Dr. George Vande Woude

Objectives: To obtain, by biochemical methods, knowledge of the virus-cell relationship established in transformations induced in experimental systems and to apply sensitive nucleic acid hybridization methods to probe for the presence of specific HSV-2 nucleic acid in cervical and prostatic carcinomas.

Major Findings: An extensive study on the conditions of mercuration of HSV DNA has led to the conclusion that the proposed scheme for the production of cell line specific hybridization probes would not be feasible. Additional studies utilizing DNA cellulose, however, indicated that this procedure was suitable and cell line specific probes which are greater than 80% specific have been produced.

The basic concept of the usefulness of cell line specific probes was tested in a series of additive hybridization experiments. The results of these experiments clearly showed that all of the cell lines tested, both morphological and biochemical transformants, shared a viral DNA fragment approximately 2×10^6 daltons in size. Studies on a series of revertants of biochemical transformants indicated that expression of viral TK is subject to positive control by a viral gene not adjacent to the structural gene for TK.

The TK region of the HSV genome becomes very stably associated with the cellular DNA and even in "revertant" clones there is evidence for the continued presence of the HSV TK gene. These conclusions are based on a study of the TK activities found in biochemical transformants, their "revertants" and morphological transformants. Evidence obtained to date suggests that all HSV-transformed cells have a common DNA sequence and the viral TK gene is part of that nucleic acid sequence.

Significance to Biomedical Research and the Program of the Institute: Sero-epidemiological studies have shown an association between HSV-2 infection and human cervical carcinoma. To further define the role, if any, of this virus in oncogenesis, these studies are to determine whether HSV-2 genetic information can be demonstrated within cervical tumor cells, and, further, to develop a better understanding of the nature of the interaction between the virus and cells which leads to their transformation experimentally.

Proposed Course: This contract was terminated on July 31, 1978.

Date Contract Initiated: May 16, 1975

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP7-1009)

Title: Interaction of Human Papovaviruses BK and JC and Simian Virus 40 with Human Vascular Endothelial Cells

Contractor's Project Director: Dr. George C. Fareed

Project Officer (NCI): Dr. George Khoury

Objectives: To study the mechanism of transformation of human vascular endothelial cells by the human papovavirus, JC; determine the genotypic and phenotypic markers of transformation by JC; and study cells chronically infected with JC.

Major Findings: Human vascular endothelial cells (HEC) transformed by simian virus 40 (SV40) and the human papovavirus, JC, have been compared to primary human umbilical vein endothelial cells with regard to their production of specific extracellular materials. The endothelial cells, whether or not transformed, release fibronectin and another prominent material labeled with ^{3}H-proline or lysine into the cell culture medium. They also release one or more species of basement membrane collagens, but no other collagens, into the medium if they are transformed. This basement membrane collagen is probably larger than interstitial procollagens and probably contains individual chains in disulfide linkage. Its behavior is consistent with a triple collagen helix containing a few pepsin or trypsin sensitive sites and some nonhelical regions.

The Southern gel electrophoretic-hybridization procedure was used to demonstrate the presence of both free and integrated BK papovaviral DNA sequences in T-antigen negative transformed human fetal brain cells. Acute infection of primary human fetal brain cells with human papovavirus BK has been shown to cause extensive cellular vascularization and destruction. A small fraction of surviving cells can be recovered by frequent medium changes. These survivors are rapidly dividing cells which form visible colonies within four to six weeks after infection. Permanent cultures can be established from these cell colonies termed BK-HFB cells. BK-HFB cells have some properties of transformed cells (rapid growth rate, increased saturation density, colony formation in soft agar) and are persistently infected with BKV. Unlike SV40 in human cells or polyoma virus in mouse cells, only a small percentage of the cells are T-antigen positive, corresponding roughly to the number of virus-producing cells in culture.

Cloning experiments have demonstrated a permanent association of BKV with the transformed cells and that a simple BKV carrier state does not exist in these cells. Twenty clones were obtained from single cells propagated in medium containing anti-BKV antiserum. Morphologically all clones appeared to be similar and to resemble the parental cells. All cloned lines, when kept in antiserum-containing medium, remained virus-free and T-antigen negative. In contrast, every clone which was grown in antibody-free medium began to release virus by three to four weeks and showed 5 to 10% T- and V-antigen positive cells.

Significance to Biomedical Research and the Program of the Institute: Papovaviruses have been shown to produce cancers in experimental animals, but the relationship of these viruses to human cancer has not been defined. The isolation of human papovavirus strains from urine, brain tissue, and certain tumors necessitates investigation of their transforming activity

in human cells and association with human disease. This project provides the opportunity to use a human diploid cell system to study the molecular interactions of viral genomic sequences with human cell DNA with respect to cell transformation and to acquire a well-characterized system for study of the relationships of papovaviruses to human neoplasia.

Proposed Course: The project will continue without change.

Date Contract Initiated: January 15, 1977

EMORY UNIVERSITY (NO1-CP4-3393)

Title: Cellular Immunity Studies to Herpes Simplex Virus-Associated Antigens in Cancer Patients and Controls

Contractor's Project Director: Dr. Andre Nahmias

Project Officer (NCI): Dr. Steven R. Tronick

Objectives: The major objectives of this contract are: (1) to provide the necessary immunological infrastructure required to evaluate any prospective HSV vaccine for human use; (2) to obtain evidence corroborating the hypothesis that HSV-2 plays an etiological role in human cervical neoplasia; and (3) to provide a better understanding of the host factors involved in primary and secondary HSV infection.

Major Findings: The primary objective of these studies was to apply assays of cellular immunity already developed, or to be developed, to obtain further evidence of an association between HSV-2 and cervical neoplasia. Secondary objectives included obtaining a better understanding of the immunology of HSV infection and of cervical cancer.

Since preliminary efforts with the technology available at the initiation of these studies failed to yield significant results, efforts were directed towards developing newer assays and attempting to understand their mechanisms, so that they could be applied to the problem with better understanding. Success with such approaches was obtained for: (1) antibody-dependent cell cytotoxicity with K lymphocytes, monocyte-macrophages and polymorphonuclear leukocytes and complement-dependent antibody cytotoxicity using HSV-infected target cells; (2) lymphocyte transformation assays, including effects of hyperthermia; (3) lymphokines; (4) rosette-forming T-cells and effect of anti-lymphocyte serum; (5) detection of immune complexes using Fc positive mouse leukemic cells and protein A-containing staphylococci; (6) adapting serological methods to demonstrate a further relation between HSV-2 and cervical cancer and the detection of circulating tumor-associated antigens. Intensive efforts to demonstrate T-cell cytotoxicity to cervical cancer cells or to HSV-early-infected or late-infected target cells failed. Some evidence was obtained of a greater lymphocyte transformation response to HSV-2 antigens in women with cervical neoplasia than controls and of

the presence of suppressive factor(s) in the sera of cancer patients. However, when various assays were applied to target cultured cervical cancer cells, no evidence of greater reactivity in women with HSV-2 infection as compared to controls was obtained. No marked cytotoxic differences were shown between women with cervical anaplasia, as compared to controls, when target HSV-2 infected cells were used.

Significance to Biomedical Research and the Program of the Institute: The evaluation of cell-mediated immune responses to HSV-2 in patients with cervical carcinoma may add further information for determining whether there is an etiological link between the virus and the neoplastic disease and on host factors involved in HSV-2 infection and disease.

Proposed Course: Studies were completed at the end of the current contract year (December 31, 1977).

Date Contract Initiated: June 28, 1974

GOTHENBURG UNIVERSITY (NO1-CP8-1020)

Title: Studies on the Biomolecular Relationship of Herpesviruses (Epstein-Barr Virus) and Cancer

Contractor's Project Director: Dr. Tomas Lindahl

Project Officer (NCI): Dr. George Vande Woude

Objectives: The molecular characterization of the Epstein-Barr Virus (EBV) genome and comparison of the latent EBV genomes present in a series of human cell lines derived from patients with various diseases associated with EBV; and the determination whether specific EBV strains are correlated with certain malignant diseases.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: Recent advances in biochemical techniques for segregating and characterizing small DNA fragments released by endonucleases provide the opportunity to assay tumor tissues for the presence of a small fraction of herpesvirus genome in the host cell genome. A major objective is the determination of etiology of diseases which so far have been shown to be associated with specific DNA viruses by serological and epidemiological techniques. The new capability to extend present knowledge to include biochemical genetic information about the relationship of specific DNA viruses to specific cancers has a high probability of contributing to cancer etiology.

Proposed Course: This project will continue without change.

Date Contract Initiated: October 31, 1977

HARVARD UNIVERSITY (NO1-CP3-3390)

Title: Oncogenic Herpesviruses in Primates

Contractor's Project Director: Dr. T. C. Jones

Project Officer (NCI): Dr. Dharam Ablashi

Objectives: To study oncogenic herpesviruses in primates.

Major Findings: A newly-isolated, highly oncogenic variant strain of Herpesvirus saimiri (HVS) was shown to produce malignant lymphoma consistently with a short incubation time in owl monkeys and common marmosets. Several strains of HVS underwent attentuation after growth in Vero cells, human cell cultures and dog fetal lung cultures. HVS was demonstrated to produce malignant lymphoma in cottontop marmosets and owl monkeys directly in contact with infected squirrel monkeys. The in vivo neutralization of HVS was demonstrated in cottontop marmosets. The variable response of owl monkeys to attenuated strains of HVS was shown not to be different in owl monkeys of karyotypes I, II, III and VI. HVS stimulated lymphocytic transformation in the African green monkey although lymphoma did not occur. New Zealand white and ACCRB inbred strains of rabbits were shown to be susceptible to malignant lymphoma and leukemia by injection with tissue culture preparations and purified HVS. HVS was not transmitted between infected and normal rabbits by intimate contact. Purified HVS M-DNA produced malignant lymphoma in marmosets (Saguinus oedipus). Immunologic studies with HVS in goats demonstrated development of neutralizing and fluorescent antibodies and complement requiring antibodies at high titers.

Antibodies to Herpesvirus ateles (HVA) were prepared in New Zealand white rabbits and goats. Studies on the pathogenesis of HVS revealed that owl monkeys were quite susceptible, developing malignant lymphoma. Cebus albifrons, Macaca fascicularis, Macaca cyclopis, and M. mulatta did not develop lymphoma or antibodies following injection with HVA. ACCRB inbred rabbits were very susceptible, developing antibodies and malignant lymphoma following injection with HVA. New Zealand white rabbits were refractory, although antibodies were developed. Day-old hamsters, mice and rats showed no signs of infection after injection with HVA. A tumor cell line was established from lymphoblastoid cells from a rabbit which developed malignant lymphoma after inoculation with HVA. Several hundred lymphoblastic cell cultures induced by HVA in rabbits have been studied ultrastructurally and no virions have been demonstrated. Two owl monkey kidney cell lines have been established in the course of these studies (OMK 210 and 637).

Proposed Course: This project was terminated on November 30, 1977.

Date Contract Initiated: June 26, 1972.

HARVARD UNIVERSITY (NO1-CP4-3299)

Title: Investigation of Cellular, Exogenous and Endogenous RNA Viral Factors in Replication and Transformation by Polyoma Virus

Contractor's Project Director: Dr. Thomas L. Benjamin

Project Officer (NCI): Dr. Robert Goldberg

Objectives: Investigate the relationship between cells transformed by DNA and RNA tumor viruses, specifically, to test the ability of various cell lines, transformed or infected by various DNA or RNA tumor viruses, to complement the growth defect of the polyoma virus mutant, NG-18.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: The study deals with an extension and confirmation of earlier observations which suggested that similar cellular functions were expressed following transformation of cells by either RNA or DNA viruses. This is important since it may help to define the intracellular events essential for cell transformation.

Proposed Course: This contract was terminated on December 31, 1977.

Date Contract Initiated: April 1, 1974

HARVARD UNIVERSITY (NO1-CP8-1005)

Title: Biomolecular Relationship of Herpesviruses (*Herpesvirus saimiri*) and Cancer

Contractor's Project Director: Dr. Bernhard Fleckenstein

Project Officer (NCI): Dr. George Vande Woude

Objectives: The viral genome structures of four different strains of *Herpesvirus saimiri* (HVS) of varying oncogenic potential will be analyzed and compared using restriction endonucleases. HVS DNA and specific DNA fragments will be tested *in vitro* for transforming potential in isolated lymphatic primate cells and *in vivo* for their potential to induce tumors.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and to the Program of the Institute: Recent advances in biochemical techniques for segregating and characterizing small DNA fragments released by endonucleases provide the opportunity to assay tumor tissues for the presence of a small fraction of herpesvirus genome in the host cell genome. A major objective is the determination of etiology of diseases which so far have been shown to be associated with specific DNA viruses by serological and epidemiological techniques. The new capability to extend present knowledge to include biochemical genetic information about the relationship of specific DNA viruses to specific cancers has a high probability of contributing to cancer etiology.

Proposed Course: This project will continue without change.

Date Contract Initiated: January 1, 1978

HEALTH RESEARCH, INC. (NO1-CP7-1062)

Title: Biochemical Mapping of the Integration Site of SV40

Contractor's Project Director: Dr. Mary Gutai

Project Officer (NCI): Dr. C. J. Lai

Objectives: Determine the nucleotide sequences at the SV40 DNA and cell DNA junction for cloned evolutionary variants of SV40 and study the recombination between SV40 and host cell DNA.

Major Findings: This contract has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: Examination of the interactions between genomes of eukaryotic cells and oncogenic viruses may identify the basic reactions of cell regulation which are deranged by the virus and result in transformation to malignancy or which control expression of viral genetic information.

Proposed Course: This project will continue without change.

Date Contract Initiated: September 30, 1977

ILLINOIS, UNIVERSITY OF (NO1-CP4-3318)

Title: Studies on the Molecular Mechanism of Carcinogenesis by Oncogenic Viruses

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. Robert Goldberg

Objectives: Characterization of the human papovaviruses, especially BK virus.

Major Findings: A new gene and gene product of SV40 has been identified and located at 0.54 to 0.59 on the genome. This gene is necessary for malignant transformation.

A detailed map of BK virus DNA has been obtained; a total of 51 restriction sites have been recognized within the BK virus genome by the combination of nine different restriction endonucleases. These fragments were mapped and oriented to one another as well as to the five fragments generated by digestion of BK virus DNA with Hind III and EcoRI.

The presence of DNA sequences analogous to BK virus DNA in the DNA of transformed cells and human tumors has been further investigated. Previous results were confirmed using a liquid hybridization technique. Blotting experiments showed the presence of hybridizing bands in the tumor DNA; however, some were also found in control (normal) DNAs.

Significance to Biomedical Research and the Program of the Institute: Studies on small icosahedral DNA viruses such as SV40 and polyoma have contributed an impressive body of information in recent years on the interactions between these viruses and cells. The genetic analysis of the genome of these viruses, by the use of mutants, may provide a major advance in the knowledge of the mechanism of cell transformation at the molecular level. These studies have recently become of great importance with the isolation of human papovaviruses, such as BK and JC viruses.

Proposed Course: Greater emphasis will be placed on the development of the BK virus biological system and determination of the presence or absence of the BK virus genome in human tumors. This project will be completed on January 8, 1979.

Date Contract Initiated: December 9, 1971

ILLINOIS, UNIVERSITY OF (NO1-CP7-1061)

Title: Integration Sites of Papovavirus Genomes in Transformed Cells

Contractor's Project Director: Dr. K. Subramanian

Project Officer (NCI): Dr. R. Dhar

Objectives: Construction of physical maps of SV40 DNA integrated in the genome of transformed cells, and determination of the nucleotide sequences at the junction of cell DNA and the integrated SV40 DNA.

Major Findings: This contract has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: Examination of the interactions between genomes of eukaryotic cells and oncogenic viruses may identify the basic reactions of cell regulation which are deranged by the virus and result in transformation to malignancy or which control expression of viral genetic information.

Proposed Course: This project will continue without change.

Date Contract Initiated: September 29, 1977

JOHNS HOPKINS UNIVERSITY (NO1-CP3-3345)

Title: Studies on Herpesvirus Antigens and Virions in Neoplastic Cells from Human Cervical Carcinoma

Contractor's Project Director: Dr. Laure Aurelian

Project Officer (NCI): Dr. George Vande Woude

Objectives: Identification of HSV-2 antigens and virions in neoplastic human cells to develop evidence for or against HSV-2 as a factor in the etiology of squamous cell carcinoma of the human uterine cervix.

Major Findings: Forty-seven HSV-2 proteins were identified in infected cells. Virus specificity was determined by: (i) increased rate of synthesis following infection; (ii) precipitation by antisera specific to viral antigens; (iii) variation in electrophoretic mobilities or rates of synthesis following infection with different HSV-2 strains. Twenty-four of these proteins (including ICP-10) co-migrate with proteins from virus purified by two cycles of centrifugation in Dextran T-10 gradients.

Correlation of AG-4 with ICP-10 was based on: (i) positive correlation between the amounts of AG-4 and ICP-10 produced at various times following sequential treatment of infected cells with cycloheximide and actinomycin D, (ii) the similar effect of HSV-2 passage history on the synthesis of AG-4 and ICP-10; (iii) ICP-10 is precipitated by AG-4 positive but not by AG-4 negative sera; (iv) following partial biochemical purification of crude AG-4, those fractions containing the AG-4 complement fixing activity differ from those without activity in that they contain ICP-10.

ICP-10 was purified by high resolution SDS-acrylamide gel electrophoresis for 15 hours and homogeneity was achieved. The ICP-10 band contained the AG-4 activity. Antisera to ICP-10 were prepared from gel segments containing the AG-4 activity. The anti-ICP-10 sera fix complement with AG-4, and stain, in immunofluorescence, cells infected with HSV-2 and sequentially treated with cycloheximide and actinomycin D. ICP-10 is

the major viral protein synthesized under these conditions and AG-4 levels are increased.

Capsids, prepared from nuclei of HSV-2 infected cells, did not contain ICP-10 or AG-4. Solubilized surface envelope proteins contain the AG-4 activity as well as ICP-10 and 12 other viral proteins.

Antibody to AG-4 is found in women with primary HSV-2 infections further pointing to the virus specificity of AG-4 antigen. AG-4 antibody is transient and disappears within 20-160 days post infection. Most women with recurrent HSV-2 are AG-4 negative. This is most probably due to the low levels of AG-4 available as immunogen and the 19S nature of the antibody.

Significance to Biomedical Research and the Program of the Institute: Considerable data has been acquired demonstrating an association between HSV-2 infection and carcinoma of the uterine cervix. In humans, it is extremely difficult to show conclusively that virus associated with neoplasms is a factor in oncogenesis and not simply a passenger. Studies conducted under this project are expected to provide some of the data required to reach a decision regarding the role of this virus in oncogenesis.

Proposed Course: Studies were completed on October 31, 1977.

Date Contract Initiated: May 5, 1971

JOHNS HOPKINS UNIVERSITY (NO1-CP4-3330)

Title: Studies on Cellular Immunity to Herpes Simplex Virus-Associated Antigens in Cancer Patients and Controls

Contractor's Project Director: Dr. Laure Aurelian

Project Officer (NCI): Dr. Steven R. Tronick

Objectives: The objectives of this contract were (1) to establish whether cell-mediated immunity (CMI) to HSV-2 antigens can be demonstrated in selected cancer patients; (2) to identify and characterize the antigens responsible for eliciting the cellular immune responses; (3) to correlate CMI responses with serological assays designed to detect humoral antibody to HSV-2 antigens; and (4) to relate the results of these studies to the clinical status of patients and controls.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: The evaluation of CMI responses to HSV-2 in patients with cervical carcinoma may add further information for determining whether there is an etiological link

between the virus and the neoplastic disease and on host factors involved in HSV-2 infection and disease.

Proposed Course: Studies were completed on October 31, 1977.

Date Contract Initiated: June 28, 1974

JOHNS HOPKINS UNIVERSITY (NO1-CP7-1022)

Title: Biomolecular Relationship of Herpesviruses and Cancer: Herpes Simplex Virus

Contractor's Project Director: Dr. Gary Hayward

Project Officer (NCI): Dr. Berge Hampar

Objectives: Identification of the minimum portion of HSV-2 DNA necessary for transformation of animal and human cells; determination of the role of virus-specified products in maintenance of the transformed phenotype; determination of the specific sites of integration of the HSV-2 DNA in the host cell genome; and preparation of HSV-2 "transforming gene" DNA probes for high sensitivity screening of transformed cell lines and tumor tissues for the presence of HSV-2 DNA or RNA.

Major Findings: This contract has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: Recent advances in biochemical techniques for segregating and characterizing small DNA fragments released by endonucleases provide the opportunity to assay tumor tissues for the presence of a small fraction of herpesvirus genome in the host cell genome. A major objective is the determination of etiology of diseases which so far have been shown to be associated with specific DNA viruses by serological and epidemiological techniques. The new capability to extend present knowledge to include biochemical genetic information about the relationship of specific DNA viruses to specific cancers has a high probability of contributing to cancer etiology.

Proposed Course: This project will continue without change.

Date Contract Initiated: September 30, 1977

KAROLINSKA INSTITUTE (NO1-CP3-3316)

Title: Studies on the Significance of Herpes-Type Viruses and RNA Viruses in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officer (NCI): Dr. Berge Hampar

Objectives: To elucidate EBV-cell-host interactions and mechanisms of cell-mediated anti-tumor immune reactions.

Major Findings: If EBV DNA linear molecules are inhibited by phosphonoacetic acid, circles can be demonstrated in producer cell lines. This suggests that producer cells may contain nucleases which degrade covalently closed EBV DNA molecules.

Circle size reflects virus strain differences and is not related to disease differences.

Cell lines with one to four EBV genome equivalents contain only integrated sequences.

Epstein-Barr virus-directed nuclear antigen (EBNA) was purified to homogeneity giving a single 49,000 molecular weight band on SDS gel.

The amount of EBNA per nucleus was directly proportional to the number of EBV DNA copies in all cell lines and somatic hybrids studied. This indicates that EBNA induction is a relatively autonomous function of the viral genome and is not subject to cellular regulating mechanisms as are other EBV products.

Persistence or loss of EBV DNA or EBNA from cells could not be associated with any specific human chromosome.

Normal B lymphocytes adsorb EBV and respond with EBNA induction, 0-cells which are C3 receptor positive adsorb EBV but EBNA formation is not induced, and Fc positive T-cells do not adsorb EBV at all.

In Burkitt lymphoma (BL) patients, there appears to be a relationship between serum antibody-dependent lymphocyte cytotoxicity titers and a favorable clinical course.

EBV DNA and EBNA positivity at the tissue level was sharply limited to and regularly associated with anaplastic nasopharyngeal carcinoma (NPC) to the exclusion of other tumors within and outside the nasopharynx, except in cases of BL.

The DNA of an EBV-like virus, Herpesvirus papio (HVP) found in baboon lymphoblastoid cells, was present both free and integrated in the cells of five producer lines. The nonproducer line appeared to contain only integrated HVP DNA. A new nuclear antigen was detected in HVP-carrying baboon cell lines.

Significance to Biomedical Research and the Program of the Institute: Investigations under this project are directed to two areas of importance to overall Program. First, the recognition that certain herpesviruses induce neoplasms in animals and that EBV and HSV-2 are associated with human neoplasms requires intensive study to provide a better understanding

of the host-virus relationship for this group of agents. Data acquired under this project contribute to assessment of the role of herpesviruses in the causation of human neoplasms. Second, the analysis of the immunological responses of the host to tumor cell surface antigens provides basic information important in approaches to control of tumor development. The project is strongly oriented to human neoplasia, using defined animal systems as required for progress in understanding the fundamental mechanisms involved.

Proposed Course: This project will continue without change.

Date Contract Initiated: April 9, 1968

LIFE SCIENCES, INC. (NO1-CP3-3205)

Title: Studies on Marek's Disease as a Model for Herpesvirus-Associated Oncogenesis

Contractor's Project Director: Dr. Meihan Nonoyama

Project Officer (NCI): Dr. George Vande Woude

Objectives: To determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease and elucidate the mechanisms of interaction between herpes and RNA viruses in tumorigenesis using specific pathogen-free avian hosts.

Major Findings: Propagation of Marek's disease herpesvirus (MDV) has been improved by using DMSO in the culture medium and infecting secondary cultures of chick embryo fibroblasts (CEF) with MDV.

No homology was detected between MDV DNA and turkey herpesvirus (HVT) DNA; however, further analysis is required. Restriction enzyme digestion of MDV DNA showed that the virus DNA has a rather complex structure.

Transformed nonvirus-producing cells, MKT, which had been established from a Marek's disease kidney tumor, contained 25 genome copies per cell. The majority of the copies exist as circular plasmid DNA. Marek's disease tumors also contained MDV genomes and virus genome transcripts.

In MKT cells at least one virus-induced DNA binding protein was observed which may play a role in transformation of cells by MDV.

Transformation of embryonic lymphocytes by infection with MDV has been partially successful.

Infection of chick factor positive CEF with MDV appeared to induce endogenous type C virus expression.

Coinfection of chickens with MDV and a high dosage of avian leukosis virus resulted in higher mortality of the infected birds as compared with birds infected with either one of the viruses, whereas Marek's disease tumor induction was suppressed by the coinfection.

Significance to Biomedical Research and the Program of the Institute: In comparison to the RNA tumor viruses, comparatively little is known concerning the role of herpesviruses in oncogenesis or their interaction with endogenous RNA tumor viruses. Certain herpesviruses have been implicated in the etiology of carcinoma, lymphoma and leukemia in different species of animals and other viruses of this group have been shown to be strongly associated with neoplasia in man. This project provides the opportunity to acquire information on one herpesvirus in relation to a malignant disease in its natural host: this may aid in understanding the role of herpesviruses in oncogenic processes in man.

Proposed Course: Studies on the interaction between MDV and other viruses as this relates to the disease process will be continued with particular emphasis on the molecular virology of the interaction between MDV and avian leukosis viruses.

Date Contract Initiated: November 1, 1968

MELOY LABORATORIES, INC. (NO1-NS7-2360)

Title: Oncogenic Potential of Herpesviruses in Primates

Contractor's Project Director: Dr. D. L. Sly

Project Officers (NCI): Mr. J. T. Lewin
(NINCDS): Dr. W. T. London

Objectives: To evaluate HVS-2 as a potential agent of cervical carcinoma using Cebus monkeys.

Major Findings: This is a collaborative project between NCI and NINCDS. Nineteen of 49 virus recipient females have developed persistent abnormal cytology. Nine have never demonstrated cytologically abnormal smears. Cytological changes identified to date are of the mild (atypia or Class II) or moderate (dysplasia or Class III) nature. Histologically, dysplasia has been identified in necropsy specimens from one virus-infected animal and in a biopsy taken from a persistently abnormal virus recipient.

The preliminary findings in the hormone study suggest that diethylstilbestrol is quite active in producing cytological abnormalities in the vaginal epithelium and that estrogens with progesterone may be reacting as cofactors with HSV-2 to produce more severe and persistent cytological changes.

Significance to Biomedical Research and the Program of the Institute: HSV-2 has been shown to be associated with invasive cervical carcinoma in humans. This project was initiated to determine whether repeated infections in a nonhuman primate that responds to infections by this virus in a manner similar to humans would result in cervical malignancy. The data acquired in this and related projects is expected to provide a better understanding of the nature of the relationship observed between HSV-2 and cervical cancer.

Proposed Course: This project is expected to continue for one more year.

Date Contract Initiated: March 30, 1972

OHIO STATE UNIVERSITY (NO1-CP8-1021)

Title: Studies on the Epstein-Barr Virus and its Association with Nasopharyngeal Carcinoma

Contractor's Project Director: Dr. Ronald Glaser

Project Officer (NCI): Dr. Berge Hampar

Objectives: Determination of the role of EBV in nasopharyngeal carcinoma (NPC) by conducting studies: (1) on infection by EBV of normal human epithelial cells, particularly nasopharyngeal cells; (2) on cellular hybridization with an HR-1 cell line containing one EBV genome per cell; (3) on transformation of normal human primary epithelial cells with EBV by a nuclear exchange procedure; and (4) on in vivo cell fusion of EBV genome positive lymphoid cells and nasopharyngeal cells.

Major Findings: This contract has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: Sero-epidemiological surveys have demonstrated a relationship between a DNA virus and cancer. These studies will provide information as to whether EBV plays a role in NPC and if the association of the EBV genome in the epithelial cells of the tumor is important for the induction of the tumor.

Proposed Course: This project will continue without change.

Date Contract Initiated: March 29, 1978

PENNSYLVANIA STATE UNIVERSITY (NO1-CP5-3516)

Title: Studies on the Oncogenic Potential of Defective Human Viruses

Contractor's Project Director: Dr. Fred Rapp

Project Officer (NCI): Dr. Brenda Gerwin

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: Studies on quantitative transformation with HSV have continued. Morphological transformation by transfection with HSV DNA fragments has been attempted but detection of transformants has been difficult. Several human osteoma clones possibly transformed by Hind III and Eco R_I fragments have been isolated and are now being cultured. A quantitative transformation assay developed by the contractor is also being used to determine whether specific growth factors, hormones or combinations of these agents potentiate herpesvirus transformation. Cyclic AMP caused a slight increase in transformation frequency while theophylline caused a marked decrease. Diethylstilbestrol has also been studied and preliminary results indicate an inhibition of HSV transformation.

Studies to further characterize a herpesvirus, HMCV, with biologic and biochemical characteristics intermediate to cytomegalovirus (CMV) and HSV, have continued. In rabbit kidney cells, the growth is similar to that of HSV-2; in human diploid fibroblasts, virus growth is like CMV. Live HMCV transformed primary human kidney and endometrial cells in vitro. Two normal human endometrial cell lines inoculated with HMCV have survived more than 10 passages.

The molecular biology of HSV DNA has been investigated using experiments that measure the terminal repetition of HSV DNA and enumerate the palindrome and palindrome-like sequences of HSV DNA. Additional studies have also been done to further characterize the DNA of HMCV. Sucrose gradient sedimentation in alkaline and neutral gradients was used to determine the size of HMCV DNA. Using the Hershey-Burgi equation, the molecular weight of HMCV DNA was calculated to be 96×10^6 daltons. The buoyant density of HMCV DNA was measured by equilibrium centrifugation in CsCl. It appears that HMCV DNA has a slightly higher buoyant density than HSV-2 DNA and is far removed from the buoyant density of CMV DNA. Thus, based on the centrifugation studies, HMCV DNA is more closely related to HSV DNA than to CMV DNA. The Eco R_I restriction enzyme cleavage pattern of HMCV DNA is entirely distinct from the cleavage patterns of other human herpesvirus DNAs on the basis of the relative number of molar and submolar bands. Preliminary RNA-DNA hybridization studies to determine the relatedness of HMCV to other human herpesviruses indicate that HMCV has less than 10% homology to HSV-1, about 30% homology to HSV-2 but no homology to laboratory adapted strains of CMV. DNA-DNA hybridization of HSV-1, HSV-2, and CMV DNAs with HMCV DNA are in progress and should provide better quantitation.

Studies on the molecular biology of varicella-zoster virus (VZV) have continued. An investigation has been initiated to analyze the DNA and proteins from in vitro passed isolates of VZV. Buoyant density centrifugation in cesium chloride with VZV DNA from two different isolates mixed in the same gradient indicates that a small but reproducible difference may exist in guanine-cytosine content. Comparative studies will continue since this is the only virion characteristic yet found that indicates that different VZV isolates are not identical.

Significance to Biomedical Research and the Program of the Institute: The observations made under this project indicate that some strains of herpesviruses commonly afflicting humans may express oncogenic properties under certain conditions. Better assay procedures for transforming potential may provide a means to assess the significance of virus strain differences within populations leading to a better understanding of the nature of the association of HSV-2 with human cancer.

Proposed Course: Investigations on herpesviruses in relation to cancer will continue.

Date Contract Initiated: October 27, 1969

SAINT LOUIS UNIVERSITY (NO1-CP4-3359)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. George Vande Woude

Objectives: This research program is aimed to achieve increased understanding of the mechanism of cell transformation by DNA tumor viruses and to apply new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer.

Major Findings: To investigate whether ubiquitous oncogenic human DNA viruses cause human cancer, tumor DNAs and RNAs were assayed for viral "transforming genes", using in vitro labeled (1-2 x 10^8 cpm/μg) viral DNA and transforming DNA restriction fragments as probes for molecular hybridization. Over 2500 human tumors were collected, and nucleic acids extracted from 800. The 29 human adenovirus (Ad) serotypes formed five distinct DNA homology groups: group A (Ad12, 18, 31), group B (Ad3, 7, 11, 14, 16, 21), group C (Ad1, 2, 5, 6), group D (Ad8-10, 13, 15, 19, 20, 22-24, 26-30), and group E (Ad4). Ads within each group are 60-100% homologous but are <20% homologous to other Ads. In vitro labeled transforming fragments of group A Ads (Ad12 strain Huie EcoRI-C, left 16% of genome) and group C Ads (Ad HindIII-G, left 7.5% of genome were prepared. With these probes, no Ad DNA sequences were detected in 88

tumors using Ad12 transforming EcoRI-C as probe, and 113 tumors using Ad5 HindIII-G. These probes could detect one copy per tumor cell of about 1-3%, or 0.5%, respectively, of the Ad genome. Therefore, these results virtually exclude an Ad etiology for these tumors. The EcoRI-C and HindIII-G probes hybridize 53-68%, and 95%, respectively, with Ads within the same group, but <10% to Ads outside the group. Thus, these data are applicable only to Group A and C Ads, respectively. It is now clear that group D Ads transform cells. A putative Ad26 transformed cell line was found to contain multiple copies of most of the Ad26 genome, and foci of cells were transformed by Ad9, 19, and 26. Transforming fragment probes for group B, D, and E Ads are being prepared to analyze human tumors.

One hundred fifty-six tumors were assayed for human papilloma virus type 1 (HPV-1 plantar warts), and 145 tumors for HVP-2 (hand warts). No viral sequences were found. The HVP-1 and HVP-2 DNAs are only 5-8% homologous, consistent with other evidence for several distinct HVPs. Fifty-three normal tissues, 166 tumors, and seven malignant human cell lines were assayed for BK virus sequences. No viral sequences were detected. The sensitivity is one copy per cell of 5-10% or 0.1 copy of 100% of the BKV or HVP genome. DNAs of 10 normal tissues and 43 tumors were assayed for JC virus sequences; six gave 10-20% hybridization.

Five groups of Ads and two groups of HPVs were defined. No evidence has yet been obtained that human Ads, HPVs, or BK virus cause tumors representing 40-50% of cancers in the U.S.A. More work needs to be done, especially with group B, D, and E Ads, with other types of HPVs, and other tumor categories. A limited number of tumor DNAs hybridized with JC virus; however, these data are preliminary and must be confirmed and extended before their significance is understood in terms of a viral etiology of human cancer.

Significance to Biomedical Research and to the Program of the Institute: This is a systematic study using the most sensitive techniques available to probe for evidence of an association between members of the adenovirus and papovavirus groups and neoplasms occurring in humans.

Proposed Course: With the development of highly sensitive and specific DNA probes, major emphasis will be placed on the analysis of human tumors for the presence of adenovirus, papovavirus, and human papilloma virus-specific nucleic acid sequences.

Date Contract Initiated: March 20, 1967

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP6-1022)

Title: Studies of Human Wart Virus in Tissue Culture

Contractor's Project Director: Dr. Magdalena Eisinger

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To determine the antigenic properties of various human wart viruses (HWV) and the relationships of HWV to benign or malignant tumors.

Major Findings: Human hand wart viruses derived from wart tissue can be isolated in tissue culture by infection of an epithelial cell line of ectodermal origin (BE cells). Six isolates have been obtained and studied.

The virions newly synthesized in tissue culture in the presence of radioactive protein precursors (^{14}C-amino-acids) have a protein composition similar to that of wart virus isolated directly from wart tissue.

Virus preparations from wart tissues consist of a heterogeneous population of particles and a similar situation occurs with viruses grown in tissue culture. When wart viruses, derived either from wart tissues or from tissue culture are purified on a CsCl density gradient and the band at density 1.34 is used to infect cells, the virus progeny is more homogenous. Cells infected with a high multiplicity of unpurified wart viruses, however, yield progeny whose particles are lighter (1.32-1.31) than the fully infectious ones, suggesting that they may be defective virus particles and may interfere with wart virus replication in vitro.

Preliminary findings with a quantitative plaque assay for infectious virus have shown that there is a "prozone" up to a dilution of 1:80 which suggests the presence of interfering viruses. Two types of plaques, "large" and "small", have been observed.

A new quantitative micro-complement fixation developed by Cikes has been adapted to studies of HWV. This method has proved to be 4-8 times more sensitive than the conventional micro-complement fixation method for the assay of HWV. The specificity of the reactions observed was verified by cross-adsorption studies.

Micro-complement fixation has also been used to study epidermodysplasia verruciformis virus and in conjunction with immune electron microscopy it has been shown that epidermodysplasia verruciformis virus is antigenically distinct from human hand and plantar wart viruses.

Studies of human sera from patients with warts compared to a "control" population have shown that 33% of patients with warts have complement-fixing antibodies to wart viruses in comparison to 18% of the "control" population. Studies using immunofluorescence have shown that 29% of human sera from patients with warts have anti-nuclear antibodies.

Significance to Biomedical Research and the Program of the Institute: In view of the prevalence of warts in the human population and the association of warts to some cancers in animals, the wart virus requires attention as a factor possibly contributing to mammalian neoplasia.

Proposed Course: This project was completed on February 28, 1978.

Date Contract Initiated: December 1, 1975

SUMMARY REPORT

C. 1. b. (5) RNA VIRUS STUDIES SECTION

October 1, 1977 through September 30, 1978

The RNA Tumor Virus Studies Section may be assigned to three main areas of investigation: replication of viruses, detection of viral information in primate tissues, and function of viral components during differentiation and malignant or autoimmune disease.

1. Replication of Viruses. Investigations on the replicative cycle of these viruses are conducted to form a basis for detection of viral information in primate, including human, tissues and for studies on the function of the virus components during pathogenesis. The replicative cycle of these viruses includes studies on the structural biochemistry of the component parts (proteins and nucleic acids), the synthetic processes by which these components are formed, and cellular mechanisms which are able to control these processes are studied. In addition, this subsection includes projects on viral genetics and host genetic control of viral replication (cellular or immune), studies on viral nucleic acid, and study of virion proteins (structural or induced on the membrane of infected cells).

A. Genetic Studies. The Rous sarcoma virus (RSV) genome has been mapped through isolation of temperature-sensitive and deletion mutants. Isolation of mutants of unrelated avian sarcoma and carcinoma viruses has been initiated for use in studies on the origin of the transforming gene. Perhaps the most significant advance in this area was the description of in vivo recombination between ecotropic and xenotropic viruses in AKR mice by peptide mapping of the several structural proteins. Correlations between thymic titers of the xenotropic and/or recombinant viruses and incidence of thymic lymphoma could be found in AKR, and possibly in HRS/J (hairless mice), emphasizing the importance of the recombinational event in the neoplastic process. The main variability between the viruses appears to be in the env gene, which appears to contain a constant and a variable region. This also confirms earlier work defining type, group, and interspecies determinants in FLV and RLV proteins. In addition to the recombinant viruses, the new amphotropic class of murine virus was isolated from wild mice. Some evidence has suggested that this class may be the parental murine type C oncornaviruses.

Regions of genome homology in murine viruses were investigated by heteroduplex mapping. Results to date suggest that most, if not all, nonhomology occurs in the env gene region, confirming the peptide mapping. In addition, it was determined that leukemia virus genomes and sarcoma virus genomes do not form heterodimers during mixed infections.

Murine chromosome 4 (linkage group VIII) was shown to contain two loci which

control virus expression or replication. Several other replication controls may also belong to this linkage group.

DNA transfection studies identified proviral integration as the probable stage of replication at which Fv-1 restriction operates. The RNA gene product was partially purified and characterized as 18-22S. Preliminary results indicated that viral protein p12 may preferentially bind to this product. In addition viral proteins gp70 and p30 were shown to be produced in significantly reduced amounts in restrictive infections. Host range conversion from N or B tropic to NB tropic was shown to result in electrophoretic differences in the p30 and Pr65 proteins between both parental types and the converted viral stocks. One peptide of the variable region of p30 may correlate with known Fv-1 genotypes.

B. Nucleic Acid Studies. The origin of replication on the RSV viral genome has been determined by nucleic acid sequencing at approximately 100 nucleotides from the 5' end. Nascent chains are initiated at the 5' end, but elongated along the 3' end. The terminal redundancy of RSV has been determined to be 21 bases; that of MoMuLV is 55-65 bases. The mechanism of jumping the gap is still under investigation.

A tridecamer deoxynucleotide complementary to a segment of the 21 nucleotide 3' and 5' reiterated sequence of RSV 35S RNA has been synthesized, purified, and is currently being tested for activity as a possible hybridization competitor and inhibitor of virus synthesis in a tissue culture system.

Full length cDNA has been synthesized in vitro, using two different experimental conditions. One group has optimized conditions allowing high yields of complete, physically intact transcripts of large polycistronic messenger RNAs to be routinely obtained. These conditions do not require the addition of high concentrations of labeled deoxyriboside triphosphates. Smaller species of DNA synthesized in the reactions in which full size copies are made do not represent break-down products, but rather DNA anticomplementary to the larger species. This DNA contains, in the proper proportions, all of the sequences expected from the full complement, but it is only 6S in size in alkaline sucrose gradients.

A second group has synthesized full length infectious DNA, using conditions requiring high concentrations of deoxyribonucleoside triphosphates and concentrations of Mg^{2+} below the total concentration of deoxyribonucleoside triphosphosphates. The infectious murine leukemia virus DNA made in vitro probably requires at least a piece of plus strand for infectivity along with a full-length minus strand. No infectivity appears if reactions are carried out with actinomycin D present. A 5' leader sequence on the glycoprotein mRNA (most of which is derived from the 3' end) is not synthesized in the presence of actinomycin D.

Functional roles of cellular tRNAs in retrovirus expression were also studied. Specific primer tRNA may be used to isolate cellular oncornavirus-specific RNAs. A poly(A)-RNA of 28S size, isolated either from

infected or uninfected cells, is capable of specifically binding primer tRNA -- proline tRNA in mouse cell preparations, and tryptophane tRNA in chicken cell preparations. The 28S poly(A)-RNA of human cells appears to bind glutamic acid tRNA specifically.

The sequence organization of RD-114 provirus in infected RD cells was shown to be not random. Endogenous RD-114 viral-specific sequences in cat cell chromosomal DNA are integrated and covalently bound. However, infective DNA can be detected only in those domestic cat or baboon cells that are releasing endogenous virus or human or dog cells releasing exogenous type C virus.

A procedure was developed to detect specific virus nucleotide sequences in cellular DNA using the formation of "R-loops." At present, the sensitivity of this procedure permits the detection of less than 0.1 ng of DNA with RNA probes of high specific activity.

The structure of proviral simian sarcoma virus (SSV-1) genes was examined in their native conformation in chromosomes of the NC-37 and A204 human cell lines, which contain 1-3 or 2-6 copies respectively. Ninety percent of the NC-37 but only 50% of the A204 integrated sequences are being actively transcribed. The concentration of viral genes was found to be identical in nucleosomal and total nuclear DNA, clearly demonstrating that the newly integrated virus DNA was organized in a nucleosomal structure in a manner analogous to that of host DNA. Since the genome was actively synthesizing viral RNA, the data further indicated that transcriptionally active regions of the genome remain associated with histone. However, the nucleosomes over the active genes were organized in a different conformation than nucleosomes formed over transcriptionally inert DNA sequences.

C. Transcriptional Control Studies. A field vole cell line (transformed by an avian oncornavirus) which reverts to the normal phenotype is being studied for transcriptional controls. The revertant cells exhibit all of the growth properties of uninfected, normal vole cells; contain the entire viral genome including the gene for malignant transformation; and, express all of the viral genes including the transforming gene sequences as RNA at levels similar to the transformed cells from which they were derived. The entire transforming gene is present and biologically active in revertant subclones indicating that the phenomenon of reversion in this cell system is not due to a deletion or point mutation in the transforming gene sequence rendering it inactive. Thus the vole cell system is unique in that it exerts post-transcriptional regulation of not only viral-structural genes but the transforming gene sequences as well.

Various inducers of differentiation of Friend leukemia cells have been shown to have differential effects on the transcription of α and β globin genes. Alterations have been defined in cell cycle-related events associated with changes in chromatin and DNA during induction of erythropoietic differentiation.

D. In Vitro Translation Studies. In vitro translation of viral mRNA has been achieved by two contractors. A model was proposed for the initiation of protein synthesis during Rauscher virus infection. This model involves a translational control mechanism operating between the gag and pol genes on full length 35S viral RNA, and a subset mRNA, which codes for the env (envelope) proteins. The translational control mechanism results in differential levels of synthesis of viral structural gag proteins and reverse transcriptase, resulting in 20 to 25 more molecules of structural gag proteins than of reverse transcriptase. The essential feature of the control mechanism may involve an occasional read-through of a ribosome termination signal (UAG codon) that follows the gag coding region. Stimulation of translation of the gag-pol "read-through" polyprotein from virion RNA of murine leukemia viruses was shown in the presence of yeast suppressor tRNA.

E. Protein Studies. Structural virion proteins were studied for biosynthesis, establishment gene order, and function during cell replication. The emphasis of most contractors is shifting from virion structural proteins per se to study of the function of these proteins during replication and transformation, and the function of other viral-coded proteins in the cell membranes of normal and of transformed cells.

MuLV ts mutants are being used to study the relationship between cleavage of precursor proteins and viral maturation. Two mutants form late budding structures at the restrictive temperature, and accumulate gag and gag-pol precursors. With shift to permissive temperature these are cleaved and infectious virus is released.

A model was developed to define the role of recombinant gp70 in transformation; the "unnatural" recombinant molecule could be the functional product of the "leuk" gene. Several new structural proteins were described, including a new envelope component [p15(e)] in RLV. This protein may be involved in activation of the complement cascade during complement virolysis. A p32 nucleic acid binding protein possessing endonuclease activity was described in avian viruses.

In the domestic cat, expression of the endogenous RD-114 viral genome at transcriptional and translational (p30) levels, but not production of isolatable virus, is enhanced in spontaneous lymphomas, sarcomas and carcinomas compared to normal tissues, regardless of FeLV status. Most lymphoma, carcinoma and sarcomas of older cats are negative for FeLV expression RNA, p30 and infectious virus.

Thymus cells of preleukemic and leukemic AKR mice express on their cell surface elevated levels of antigens associated with the murine leukemia virus (MuLV) proteins gp70 and p30. The gp70 antigenicity was contained in a 70,000 dalton polypeptide that corresponds to the viral envelope protein, while the p30 antigenicity was contained in two polypeptides of 85,000 and 95,000 daltons that correspond to glycosylated forms of the polyprotein product of the gag gene.

FOCMA antibody absorption results suggested that FOCMA antigen is a common cat tumor antigen whose expression is independent of FeLV/FSV infection; however, this antigen may not be identical in all transformed cells.

2. Detection of Retroviral Information in Primate Tissues. Several leukemic cell lines have been established and characterized. Several new marker proteins for lymphocytes and leukemic cells have been identified. Hodgkin's histiocytic cell lines have also been established and characterized. Type C virus particles were detected by electron microscopy in 7 of 19 human placental trophoblast layers.

Reverse transcriptase assays have shown activity in fresh human placentas, fresh CLL and CML leukemic cells, and Hodgkins cell cultures. The enzyme from leukemic cells has been purified and enzymatically characterized.

Immunological studies suggest a cross reaction in proteins of particles from human histiocytic cultures and structural proteins and reverse transcriptases of several primate viruses. A reactivity between a minor gp71 determinant of FLV and a 55,000 dalton protein found on CGL leukemic cells is being investigated.

Several new gibbon leukemia viruses have been isolated and characterized. In addition, a new type D virus from squirrel monkeys was isolated. Immunological studies on host response to these infections are being pursued.

3. Function of Viral Components During Infection and Cellular Differentiation or Transformation. The other area of technology application, study of the function of the various viral expressions during normal growth and differentiation and during malignant transformation, has received increased emphasis over the past year. The reciprocal roles of infections, transformation, and differentiation are being actively pursued.

Leukemic blood cells from AKR/J mice are much more sensitive to phytohemagglutinin (PHA) than normal lymphocytes; i.e., they require less time and a twenty to one hundred-fold lower concentration of PHA for optimal stimulation. Optimal PHA concentrations for normal lymphocytes are inhibiting to leukemic cells as determined by both sterol synthesis and DNA synthesis. Inhibitors of sterol synthesis abolish PHA-activated DNA synthesis in both normal and leukemic lymphocytes. Thus, sterol synthesis may be an important event in cell proliferation. Apparently viral budding takes place at cholesterol-rich sites of the cell membrane.

Relationships between terminal transferase activity, or cystathionase activity and transformation or differentiation are being established during transformation of murine lymphocytes by Abelson virus.

The effect of RSV infection on differentiation of chick myoblasts is being studied using temperature-sensitive mutants. Synthesis of myosin and acetyl cholesterase has been associated with cessation of DNA synthesis unrelated to cell fusion to myotubes. Viral replication in mature myotubes is decreased, although the amount of unintegrated virus-specific DNA was

the same, or slightly higher, as in infected fibroblasts. The fetal form of leucine amino-transferase isozyme is expressed during RSV transformation of myoblasts and chondrocytes. Hyaluronic acid production mimics the differentiated state during chondrocyte transformation. An imcompatibility between the differentiated and transformed state was postulated.

The increased expression of viral antigens on the surface of thymus cell is correlated with an increased production of infectious ecotropic and xenotropic MuLV in the AKR thymus. During aging the percentage of cells producing ecotropic MuLV increases ten-fold, while the percentage of cells producing xenotropic MuLV increases one hundred-fold. These changes correlate with appearance of recombinant viruses and leukemic cells. The expression of these viral-coded proteins on the cell surface of thymocytes varies both quantitatively with the age of the mouse and qualitatively with the cellular populations that express these antigens.

CONTRACT REPORTS

RNA VIRUS STUDIES SECTION

Dr. Wilna A. Woods

AGRICULTURE, DEPARTMENT OF (Y01-CP4-0214)

Title: Inheritance and Oncogenicity of RNA Tumor Viruses

Contractor's Project Director: Dr. L. B. Crittendon

Project Officer (NCI): Dr. John Stephenson

Objectives: These studies make use of inbred chicken lines to define the genes involved in controlling expression of the infectious endogenous type C RNA chicken viruses (RAV-0) and will determine whether the RAV-0 virus is oncogenic.

Major Findings: Over the past two generations 13 lymphoid tumors were observed in inbred lines and crosses. No lymphoid tumors were observed in a third generation which is now 8 to 10 months old. Two transplantable tumor lines were developed from line 7_1 chickens with lymphoid neoplasms. One of these was a T-cell tumor in contrast to lymphoid leukosis which is a B-cell tumor. The other transplant line has not been characterized for cell type as yet.

Histopathological examinations of the tumors suggested that two types were occurring. One was typical of lymphoid leukosis with rather large lymphoid cells of a uniform type and size. These birds all had gross involvement of the bursa of Fabricius. The other type of tumor had a rather pleomorphic cell type not typical of lymphoid leukosis. The two transplantable tumors were derived from these birds which had no gross involvement of the bursa of Fabricius.

Two experiments involving inoculation of chicks with RAV-0 derived by cultivating tumor cells with line 15_B cells were completed and no tumors attributable to the viral inoculum were observed. These tumors were therefore not induced by exogenous lymphoid leukosis viruses, but no positive evidence for an etiologic role of RAV-0 was obtained.

Significance to Biomedical Research and the Program of the Institute: It is increasingly apparent that many vertebrates, including primates, carry information for complete RNA tumor virus expression, including oncogenic potential, in their genomes. The endogenous origin of this viral information changes our concepts of the control of at least some types of cancer from methods which eliminate or control exogenous viruses to mechanisms which can suppress endogenous viral information. These studies provide another experimental system, in addition to the mouse, where the genes controlling production of the endogenous type C virus will be defined. They will provide another system for defining the role of the endogenous virus in naturally occurring neoplasms.

Proposed Course: Continue to study the influence of endogenous and exogenous RAV-0 and the presence of natural gs antigen on the incidence of lymphoid leukosis in adult chickens.

Date Contract Initiated: July 1, 1974

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP4-3380)

Title: Host Restriction of Friend Leukemia Virus

Contractor's Project Director: Dr. Ruy Soeiro

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To elucidate the molecular mechanism of host restriction of Friend leukemia virus. Specific steps in viral replication will be analyzed to determine those which are affected by the Fv-1 gene restriction. Studies focus on (1) restriction of transcription of viral RNA, (2) failure of stable integration of viral DNA and (3) inhibition of translation of viral proteins.

Major Findings: Viral protein synthesis was studied by immunoprecipitation of virus-specific proteins in infected cells. Restrictive infections showed drastically reduced amounts of viral protein, but what did finally appear was parental in type.

Electrophoretic differences previously shown in viral p30 structural proteins were also shown for the precursor protein (pr65) for the two virus types (N- and B-tropic). The p30 of N- and B-tropic virions differed mainly in minor oligopeptides. On conversion of B- to NB-tropism, an alteration only of this minor tryptic peptide was found.

Structural studies of the genome were carried out using RNase T_1 oligonucleotide fingerprinting. These studies revealed only minor changes in genome after B- to NB-host range conversion.

Significance to Biomedical Research and the Program of the Institute: Genetic susceptibility to cancer in humans is demonstrated by many examples. A partial listing of apparently recessive conditions includes those associated with visible chromosomal disorders. Downs syndrome children have a 10-20 fold increase in incidence of leukemia. Other trisomic states (trisomy D and Kleinfelter's syndrome) also have an increased incidence of leukemia. Xeroderma pigmentosum, and autosomal recessive inherited syndrome, is associated with a high incidence of skin cancer. Fanconi's syndrome, an autosomal recessive trait, increases risk of leukemia in both homozygotes and heterozygotes. Familial cancers with apparent dominant gene(s) resulting in a highly penetrant genetic predisposition to cancer include the neurofibromatoses, retinoblastoma and other embryonal tumors, pheochromocytoma, and polyposis of the colon. Common cancers including breast, uterine, gastric and lung cancers all appear in increased incidence in some families.

The availability of the murine oncornavirus system, with congenic lines of mice differing in specific loci known to be associated with resistance or permissiveness to leukemia virus replication, serves as an excellent model to study genetic control of tumor virus replication.

Proposed Course: This contract will terminate on December 31, 1978.

Date Contract Initiated: June 25, 1974

CALIFORNIA INSTITUTE OF TECHNOLOGY (NO1-CP4-3306)

Title: Electron Microscope Studies of Tumor Virus Nucleic Acids

Contractor's Project Director: Dr. Norman Davidson

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To define the structure of oncornavirus RNA and the position of the integrated viral genome in cellular DNA by biochemical, biophysical and electron microscopic techniques.

Major Findings: The dimer structure previously observed for the 50-70S RNA extracted from RD-114, endogenous baboon, woolly monkey viruses, and from several murine viruses were observed in several additional murine virus RNAs and in that of one avian virus - the reticuloendotheliosis virus. The universal occurrence of this structure indicated that it has important functional significance.

The dimer RNA extracted from virions in several cell lines producing both a murine leukemia helper virus and a defective murine sarcoma virus (with a shorter RNA genome) was either leukemia-leukemia or sarcoma-sarcoma; there were no heterodimers.

The heteroduplex method of mapping regions of sequence homology and non-homology between related tumor virus genomes was applied to a number of transforming viruses, especially to the transforming recombinants (the MCF viruses) between some common leukemia viruses and endogenous xeno-tropic viruses. The main, perhaps only, region of non-homology appeared to be in a region probably corresponding to the region coding for envelope protein.

Procedures for the direct enrichment of tumor virus genomes integrated in chromosomal DNA were further improved and were being tested.. Formamide reassociation conditions for efficient formation of R-loops were defined.

Significance to Biomedical Research and the Program of the Institute: Successful application of the proposed methodology will make it possible to determine precisely where the cancer virus genome is integrated into the transformed cell DNA; i.e., whether present at one site or at multiple sites;

whether different defined portions of the genome are in different portions of the cell. The approach used permits direct visualization of the degree of homology between viral RNAs and is particulary important for the comparative study of endogenous viral information and exogenous virus infection in relation to the transformation process and tumor development.

Proposed Course: The contractor will (1) study, by electron microscopy and other physical methods, the structure of the high molecular weight RNA extracted from RNA tumor viruses. The nature of the bond between the two monomers will be determined to find out it there is a cross-linker molecule (either a protein or a small RNA). (2) Develop a heteroduplex method, using a cDNA transcript from one viral RNA, to map the regions of homology and of nonhomology between the RNA molecules of related tumor viruses. The sequence relations between different related tumor virus RNA's will be systematically studied.

Date Contract Initiated: March 1, 1974

CALIFORNIA INSTITUTE OF TECHNOLOGY (NO1-CP7-1004)

Title: Restriction Endonuclease Analysis & EM Studies of Integrated Viral Genomes

Contractor's Project Director: Dr. Norman Davidson

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To map the site (or sites) on the viral genome where integration occurs into a site (or sites) on the cellular genome; to determine the effect of integration at specific sites on the outcome of the infection and control of virogene expression.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: There is abundant evidence that integration of the viral genome into the host genome is a prerequisite for establishement of a productive retroviral infection in a given cell line, for the occurrence of leukemia in vivo, and for transformation in tissue culture and sarcomas in vivo. Different cell lines, which contain integrated tumor virus sequences, may or may not produce virus. Proviral DNA from some of these lines is infective, other proviral DNA is not. A difference in the way in which the viral genome is integrated may account for these differences. Therefore an understanding of the sequence organization of the integrated viral genome with respect to the host genome is essential for an understanding of the factors that control viral gene expression.

Proposed Course: Restriction endonuclease digestion of cellular DNA and analysis of various fragments for integrated sequences by hybridization

techniques and heteroduplex mapping will be applied to elucidate effect of the site of integration of RD-114 and baboon endogenous virus on expression of virogenes. Infectivity studies of defined fragments of avian retroviral genomes will be conducted. The structure of the integration site of avian tumor viruses will be studied by electron microscope techniques.

Date Contract Initiated: September 1, 1977

CALIFORNIA, UNIVERSITY OF, BERKELEY (NO1-CP5-3537)

Title: Transformation of Differentiating Cells

Contractor's Project Director: Dr. G. S. Martin

Project Officer (NCI): Dr. Charles Sherr

Objectives: To investigate the relationship between transformation by RNA tumor viruses and the differentiation of the target cell.

Major Findings: A temperature-sensitive mutant of Rous sarcoma virus, tsLA29 was used to study the effect of viral transformation on the differentiation of chick myogenic cells *in vitro*.

The activity of another myogenic marker, acetylocholinesterase (ACHE) has now been measured in cultures transformed by tsLA29. The specific activity of this enzyme was greatly stimulated by the shift from 35°C to 41°C. The increase in this activity was evident both in the cell homogenate and in the nutrient medium. Cells infected with the wild type virus, Prague RSV, yielded only background levels of enzyme activity at either temperature.

The replication of RSV in mature, post-mitotic myotubes was also examined. Myotubes (in cultures freed of fibroblasts by treatment with cytosine arabinoside) were infected with various strains of RSV. The yield of infectious virus was reduced more than 200-fold as compared to parallel cultures of infected fibroblasts. The synthesis of virus-specific DNA in myotubes was also examined. The amount of unintegrated virus-specific DNA was found to be approximately the same as in infected fibroblasts. Thus the block in replication does not appear to result from a block in viral DNA synthesis.

Significance to Biomedical Research and the Program of the Institute: Many people refer to cancer as a disease of cell differentiation. The degree to which a tumor cell retains histological evidence of differentiation is often used by pathologists as an indication of its state of malignancy. Furthermore, if tumor cells retain some of the growth characteristics, such as hormone dependence, of the parent cell, these properties can be of great importance in therapy. Often, if malignant cells undergo terminal differentiation, their malignant characteristics are lost. Thus, this study of viral transformation of differentiating cells represents an approach which may be relevant to both diagnosis and therapy of cancer.

Proposed Course: This contract will terminate December 31, 1978.

Date Contract Initiated: July 1, 1975

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CP3-3242)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Thomas G. Kawakami

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To detect, isolate and characterize type C viruses from spontaneous malignancies of primates and humans.

Major Findings: Efforts were continued to study the biology of the infectious primate type C virus in gibbons and to define these agents based on the examination of the viral genome. Pathogenicity studies of the virus in gibbons were continued. Gibbons experimentally and naturally infected with the agent were monitored for clinical, virological, and immunological changes. Antibody positive and uninfected gibbons were experimentally inoculated with cell-free virus preparation at multiple sites and were evaluated for the pathogenicity of the virus. An unexposed gibbon inoculated IP, IM, and IBM with cell-free virus (9×10^3 TCIV) developed viremia without detectable immune response. The animal developed leukopenia following the inoculation, then showed an increased leukocyte count. The count rose from 2,000 cells/mm^3 to 39,500 cells/mm^3 after four weeks. The granulocytes showed a left shift with increased numbers of immature cells. The alpha globulin had become elevated similar to other pre-crisis gibbons that eventually developed myeloproliferative disease. The antibody positive gibbon inoculated with the same inoculum developed viremia without any secondary immune response. The animal was leukopenic but did not develop any clinical symptoms. One gibbon inoculated only by IP and IM route initially developed viremia but secondarily developed persisting immune response without detectable viremia. These results indicated that gibbon type C virus was oncogenic in young gibbons and that the route of inoculation could enhance the onset of the disease.

Molecular hybridization was used to differentiate and characterize the viruses isolated from naturally infected gibbons. The gibbon virus genome of either experimentally or naturally transmitted virus remained stable as determined by cDNA-70S RNA hybridization and T_m analysis. Based on the stability of the viral genome on passage through unrelated gibbons, the four gibbon viruses which were isolated from animals housed at the SEATO Laboratory were distinct substrains. This indicated that the viruses isolated from animals in this colony were not from a single common infection.

The distribution of proviral DNA in leukemic gibbons and the number of proviral copies per infected cell depended on whether the cells were from "target" organs and on when the infection occurred during the development of the animal. Preliminary examination of human tumors for gibbon proviral sequence suggested that the spleen of patients with lymphocytic leukemia might contain a small DNA sequence that was homologous with one of the gibbon virus substrains. Tumor tissues from two Old World monkey species which were examined for infectious virus following tissue culture propagation and cocultivation were found to lack evidence for the presence of any detectable oncogenic agent. The gibbon colony increased with five births.

Significance to Biomedical Research and the Program of the Institute: This gibbon colony offers a unique opportunity for the study of type C virus-associated leukemias spontaneously occurring in an ape. The virus materials obtained are important as probes for related components in human cancer cells. Comparative study of normal animals with those that develop disease provides opportunity to obtain some insight into host factors related to expression of disease.

Proposed Course: (1) Continue efforts to detect and isolate oncogenic agents from spontaneous tumors of primates and humans utilizing sensitive in vitro techniques. (2) Characterize newly isolated primate viruses and virus-like particles in human tumors and placentas. (3) Expand epidemiologic studies on primate viruses to determine animal carriers of the primate viruses. (4) Continue pathogenesis studies with primate viruses in primate species with special emphasis on the gibbon viruses. (5) Continue studies on the immune response (humoral and cell-mediated) in gibbons.

Date Contract Initiated: November 1, 1969

CALIFORNIA, UNIVERSITY OF, SAN FRANCISCO (NO1-CP4-3381)

Title: Isolation of Human Xenotropic Viruses

Contractor's Project Director: Dr. Jay A. Levy

Project Officer (NCI): Dr. Takis Papas

Objectives: (1) To establish placental cell lines from patients with systemic lupus erythematosus (SLE) and lymphocyte cell lines from patients with acute leukemia. Cocultivate the human cells with cells from different animal species to attempt virus recovery. (2) To characterize the reverse transcriptase isolated from human placentas.

Major Findings: Cell lines were derived from three additional placentas obtained from patients with systemic lupus erythematosus (SLE). Cells inoculated with acute myeloblastic leukemia cells were also cultivated.

Extracts from over 75 placentas contained RNA-directed DNA polymerase (reverse transcriptase)(RT) activity in fractions having a density of

1.15 g/ml and 1.24 g/ml. Peaks of activity at 1.12 g/ml and 1.20 g/ml were also detected in several extracts of human placentas. The RT activity was demonstrated consistently with the poly rCm·oligo dG_{12-18} template. It did not result from terminal transferase or a DNA polymerase. This activity at 1.15 g/ml converted to a density of 1.24 g/ml after heating. Some experiments suggest the 1.12 g/ml converts to the 1.20 g/ml peak on heating. Endogenous activity was also observed in the placental extracts at the active fractions listed above. The product of the endogenous reaction had characteristics of a RNA-DNA hybrid structure. The reverse transcriptase activity was not inhibited by antisera to several known mammalian retroviruses. Because of a preference of the human placental enzyme for magnesium, the human placental virus-like particles observed by electron microscopy were most likely type D viruses.

Proposed Course: This contract terminated July 27, 1978

Date Contract Initiated: June 27, 1974

COLUMBIA UNIVERSITY (NO1-CP6-1008)

Title: Transcriptional Regulation of Eukaryotic Gene Sequences

Contractor's Project Director: Dr. Paul A. Marks

Project Officer (NCI): Dr. David Troxler

Objectives: The overall objectives of this contract are to investigate the regulation of gene transcription in oncogenic virus-infected murine cells which have the potential for erythroid differentiation, specifically, to define factors which affect the transcription of structural genes characteristic of the differentiated state of Friend virus-infected murine erythroleukemia cells, namely, globin genes. The expression of differentiated transcription programs in both fetal mouse erythroid cells as well as in the Friend virus-infected murine erythroleukemia cells will be characterized.

Major Findings: The molecular portion of the bisacetamides (acetylated diamines) which provided maximal activity was the amide bond and the optimal chain length for related inducing compounds was 5 to 6 methylenes separating functional groups. Introducing rigid constraints on the methylene bridge between the acetamide groups eliminated inducing activity. Introduction of an unsaturated double or triple bond into the methylene chain did not alter inducing activity. Attachment of the inducer, hexamethylene bisacetamide, to a molecule which could not penetrate the plasma membrane, e.g. glutathione, abolished inducing activity. Attempts to potentiate or inhibit inducing activity employing inonophores were without detectable effect on inducing activity.

Attempts to mimic the action of inducer by manipulating (elevating) cyclic AMP levels with phosphodiesterase inhibitors was associated with weak to moderate induction.

In the presence of optimal concentrations of inducer, the compound entered the cell at a linear rate for 10 hours after initiation of culture; intracellular accumulation reached a plateau by 24 hours and remained unchanged between one and five days. When cells were transferred to inducer-free medium, the concentration/ μg protein decreased with each subsequent cell division.

At suboptimal concentrations of inducer, the final level achieved in the cell population was reduced proportionately. Under such conditions the proportion of cells committed to differentiate was also reduced proportionately. However, the extent of hemoglobin accumulation per cell was unaffected by suboptimal conditions. Cells resisting the differentiation-inducing effect of suboptimal concentration of inducer did not represent a unique population of cells more resistant than the average. Exposure of MELC to UV irradiation and suboptimal concentrations of inducer markedly enhanced the rate of induced differentiation over that seen with either treatment alone. Uptake of inducer was virtually complete by the time cells became committed to differentiate. The proportion of mouse erythroleukemia cells (MELC) that were committed to differentiate was dependent upon both the concentration of inducer and the duration of exposure to the inducing agent. In synchronized cultures, cells with or without inducer proceeded through S, G_2, and M phases in synchronous fashion and with the same transit times. Thereafter MELC cultures with inducer remained arrested in G_1 for a prolonged time.

Globin structural genes in both uninduced and induced cells appeared to be in an equivalent configurational state with respect to DNAse II sensitivity and in a transcriptionally "active" configuration.

In order to exploit a genetic approach to the problem of induced gene transcription, inducer-resistant variant MELC were isolated.

Significance to Biomedical Research and the Program of the Institute: This contract should provide better understanding of the control of transcription in oncogenic virus-transformed cells. More specifically, these investigations should lead to the identification of chemicals that can, in a predictable manner, alter the expression of genes in virus-transformed erythroleukemia cells and provide an understanding of mechanisms involved in inducing virus-transformed cells to differentiate and lose oncogenic properties.

Proposed Course: This contract will terminate December 31, 1978.

Date Contract Initiated: June 27, 1976

COLUMBIA UNIVERSITY (NO1-CP7-1016; successor to NO1-CP6-1010)

Title: Replication of Oncogenic RNA Viruses and its Relation to Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officer (NCI): Dr. Takis Papas

Objectives: To explore the possible involvement of RNA tumor viruses in human neoplasia and to exploit any leads that emerge for use in the diagnosis and therapy of cancer.

Major Findings: The conditions required to synthesize physically complete DNA transcripts of RNA using purified AMV reverse transcriptase were defined and simplified. The structure of viral genes in the chromatin was examined. Further characterization of the particles previously identified in human breast cancer was achieved. The reverse transcriptase from these particles was purified and antigenic cross-reactivity with the reverse transcriptase found in the Mason-Pfizer monkey virus was demonstrated.

Plasma levels of a 52,000 dalton viral glycoprotein (gp52) of the murine mammary tumor virus (MMTV) correlated with the presence of primary of metastatic mammary tumors in mice. Serial assay for gp52 could anticipate the onset of relapse following surgical removal of the tumor. Finally, the early response of gp52 provided a rapid prognostic indicator of the eventual therapeutic outcome of adjuvant chemotherapy. New drug combinations involving 5-fluorouracil and pyrimidine ribonucleosides which possessed remarkably superior therapeutic effestiveness were identified. The mechanism of action was examined.

An extensive attempt was instituted to extend the findings with the mouse mammary tumor model to human breast cancer. As a prelude to looking for a corresponding diagnostic antigen in the plasma of patients with disease, the presence of an antigen cross-reactive with gp52 was demonstrated in human tumor cells using the immunoperoxidase indirect technique. This antigen was not detected in normal breast tissue or in benign (fibrocystic disease or fibroadenoma) lesions of the human breast.

Significance to Biomedical Research and the Program of the Institute: A systematic molecular biological study is being pursued to determine the role of viruses in the genesis of human cancer. Studies demonstrated the presence in human cancers of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. New approaches to the study of virus-cell relationships were devised. The contractor developed data which are highly provocative, and his pioneering advances provide a foundation for future investigation leading to an understanding of the virus-cell relationship as it applies to human cancer.

Proposed Course: The contractor will produce intact cDNA transcripts in vitro of the complete oncornaviral genome, and use these complete copies for restriction enzyme mapping of the viral genome.

The role of nucleoproteins in the regulation of the transcription of oncornaviral genomes will be studied.

The structure of initiation sites of avian oncornaviral RNA will be identified. Diagnostic and/or prognostic techniques will be developed for identifying oncornaviral translation products in body fluids and/or tumor materials of mammalian (including human) species.

The virus-like components of the particles found in human malignancies will be characterized biochemically and/or immunologically, and their relationship to known mammalian oncornaviruses will be determined.

Date Contract Initiated: October 29, 1969

COLUMBIA UNIVERSITY (NO1-CP7-1055)

Contractor's Project Director: Dr. Ramareddy V. Guntaka

Project Officer (NCI): Dr. John Stephenson

Objectives: To study the integration of avian sarcoma virus (ASV) DNA in permissive and nonpermissive eukaryotic cell genomes by identifying the chromosome(s) on which the ASV genome is integrated and to isolate the nucleotide sequences that flank the integrated genome.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and to the Program of the Institute: Oncogenic viruses interact with cells at several levels; the intracellular microenvironment at the particular site(s) of interaction may influence the outcome of the infectious process. For example, integration of the viral genome in the cellular DNA at particular sites may determine whether viral replication proceeds and/or the cell transforms. This project to examine the interactions between genomes of eukaryotic cells and oncogenic viruses may identify the basic reactions of cell regulation which are deranged by the virus resulting in transformation to malignancy or which control expression of viral genetic information.

Proposed Course: The contractor will identify viral sequences on chromosomes by hybridizing ASV-specific cDNA containing 5-methyl dCTP to chromosome smears and utilizing specific anti 5-methyl cytidine serum to locate the sequences by indirect immunoperoxidase technology. Purification of these cellular sequences involved in viral DNA integration will be achieved by R-loop techniques. The flanking host sequences will be identified.

Date Contract Initiated: September 15, 1977

ENERGY, DEPARTMENT OF (Y01-CP6-0500)

Title: NCI-ERDA Viral Carcinogenesis Program: Regulation of Gene Expression

Contractor's Project Director: Dr. Ray A. Tennant

Project Officer (NCI): Dr. Charles Sherr

Objectives: To study the regulation of tumor virus expression, focusing on molecular mechanisms in induction and repression of tumor virus genomes. Emphasis will be placed on defining the low molecular weight RNA components of viruses and their relation to cellular RNAs.

Major Findings: Fv-1 permissive cells infected at MOI of 1 or below yielded infectious Hirt supernatant DNA whereas Fv-1 restrictive cells persistently yielded non-infectious Hirt supernatant DNA. When the restriction was overcome by MOI of 5 or above, infectious Hirt supernatant DNA could also be obtained from Fv-1 restrictive cells. Preliminary results showed preferential binding activities of viral p12 to Fv-1 gene specified RNA which were consistent with the MuLV tropism and the Fv-1 specificity.

Progress was made with primer binding sequences by the use of cellular oncornavirus-specific RNAs. Poly(A)$^+$ RNA preparations from virus-infected mouse cells were found to contain the $tRNA^{pro}$ binding sequence whereas poly(A)$^+$ RNA preparations from uninfected cells contained no such sequence. A poly(A)$^-$ RNA of 28S size, isolated either from infected or uninfected cells, is capable of binding primer tRNA specifically -- proline tRNA in mouse cell preparations, and tryptophan tRNA in chicken cell preparations. The 28S poly(A)$^-$ RNA of human cells appeared to bind glutamic acid tRNA specifically.

Significance to Biomedical Research and the Program of the Institute: This project is focused on molecular mechanisms involved in viral carcinogenesis. The problem is being investigated in terms of enzymology, immunology, cell biology, and control of gene expression primarily in the mouse leukemia system. The findings are being carried over into work with human tumor cells in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: One of the principal objectives of this activity is to define, in molecular terms, the cellular mechanisms by which expression of endogenous RNA tumor virus genomes is regulated. The long range goal is the isolation and characterization of the presumed cellular "repressors" which act in this regulation.

There are two levels of virus-host cell interaction of primary importance. One is at the genomic level where the critical impact of carcinogenic insult

takes place; and the other at the level of the cell membrane where RNA tumor viruses first come into contact with the cell and where major characteristics of neoplastic transformation are expressed. Particular efforts will continue to focus on the host cell factors involved in this virus-cell interaction.

Date Contract Initiated: July 1, 1973 (Separate Viral Oncology contract, September 1, 1972)

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CP6-1009)

Title: An Immunogenetic Analysis of Mouse Leukemia Viruses

Contractor's Project Director: Dr. Robert C. Nowinski

Project Officer (NCI): Dr. Peter Fischinger

Objectives: To isolate and characterize MuLV produced by AKR leukemias, study expression of endogenous MuLV in high leukemic mouse strains, define genetic control of expression of MuLV, isolate and characterize preleukemic lymphocytes from thymic tissue.

Major Findings: The age-related increased expression of viral antigens on the surface of thymus cells of AKR mice was correlated with an increased production of infectious ecotropic and xenotropic MuLV in the thymus. During aging the percentage of cells that produced ecotropic MuLV increased ten-fold, while the percentage of cells that produce xenotropic MuLV increased one hundred-fold.

Viral proteins expressed on the surface of MuLV-induced leukemias were examined by serological and biochemical criteria. The 95,000 dalton polyprotein ($gP95^{gag}$) contained antigens of p30, p12, and p10, while the 85,000 dalton polyprotein ($gP85^{gag}$) contained antigens of p30 and p12, but not p10. Both $gP95^{gag}$ and $gP85^{gag}$ contained glucosamine, but not fucose. Pulse-chase analysis of virus produced after a cell surface-labeling demonstrates that gp70, but not $gP95^{gag}$ or $gP85^{gag}$, was incorporated into progeny virus. Instead the $gP95^{gag}$ and $gP85^{gag}$ polyproteins were shed from the cell surface into the extracellular fluid, where they were susceptible to proteolytic cleavage.

Metabolic labeling of cells, in conjunction with pulse-chase studies, demonstrated that the viral structural proteins were produced through the processing of polyprotein precursors: (A) The gag gene products were formed through three major intracellular polyproteins of 75,000 daltons ($Pr75^{gag}$), 65,000 daltons ($Pr65^{gag}$), and 55,000 daltons ($Pr55^{gag}$). Each of these precursors was labeled in the pulse period, and dmonstrated a rapid turnover during the chase. The cell surface polyprotein $gP95^{gag}$ was labeled immediately following the pulse, but in comparison to the intracellular precursors, was processed at a relatively slow rate. There did not appear to be an obvious precursor/product relationship between either $gP95^{gag}$ or

$gP85^{gag}$ and the intracellular polyproteins. However, a precursor/product relationship was found for $gP95^{gag}$ and $gP85^{gag}$. $gP85^{gag}$ appears late in the chase period and was derived by sequential modification of the $gP95^{gag}$ polyprotein. (B) The env gene products were formed through a single 90,000 dalton glycoprotein precursor ($Pr90^{env}$). This precursor was slowly processed by proteolytic cleavage and produced the gp70 and p15(E) envelope proteins. (C) The pol gene products were formed through precursors of 180,000 daltons ($Pr180^{gag-pol}$), 120,000 daltons ($Pr120^{pol}$), and 110,000 daltons ($Pr110^{pol}$). The $Pr180^{gag-pol}$ polyprotein contained antigens of p30 and polymerase, whereas the $Pr120^{pol}$ and $Pr110^{pol}$ polyproteins contained antigens of polymerase, but not p30. Neither the polymerase nor its precursor polyproteins were detected on the cell surface.

Peptide maps of the intracellular and membrane polyproteins demonstrated a close structural relationship between the $gP95^{gag}$, $Pr85^{gag}$, $Pr75^{gag}$, and $Pr65^{gag}$ polyproteins. Furthermore, although $gP95^{gag}$ and $gP85^{gag}$ differed in their serological reactivity with anti-p10 serum, peptide mapping demonstrated that both polyproteins contained p10. It was concluded, therefore, that the $gP95^{gag}$ and $gP85^{gag}$ polyproteins represented conformational (and serological) variants of a single polypeptide.

Significance to Biomedical Research and the Program of the Institute: The pathogenic mechanism of viral-induced neoplastic transformation in mice known to be carrying vertically transmitted oncogenic viruses has been difficult to define. It is important to isolate the influence of various genetic controls on virus expression on target cells and on production of the several viruses during leukemogenesis. Identification of virus-coded marker antigens on cells destined to become neoplastic enable detailed studies on the pathogenesis of "spontaneous" leukemia.

Proposed Course: The following studies will be continued: isolation and characterization of the AKR leukemia virus complex by physical, immunological, and biological techniques; definition of the genetic control of virus expression by use of inbred congenic lines; and isolation and characterization of preleukemic lymphocytes.

Date Contract Initiated: November 1, 1975

JACKSON LABORATORY (NO1-CP3-3255)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host-Gene Control of their Expression

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and resistance to cancer and the RNA tumor viruses. The Jackson

Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on type C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings: Various recombinant inbred strains were utilized successfully to map a number of genetic loci: Lps, a gene determining responsiveness to bacterial lipopolysaccharides (Chromosome 4); Lyb-2, a B-lymphocyte alloantigen locus (Chromosome 4); and Mls, the major lymphocyte stimulating locus (Chromosome 1).

Genetic studies of the idiotype of BALB/c myeloma protein S117 suggested complex pseudo-allelic relationships between different Ig-1 haplotypes that allowed the expression of the same genes in allelic and in non-allelic fashion.

Xenotropic murine leukemia virus cell surface antigen expression (XenCSA) in DBA/2 mice was shown to be regulated by a gene linked to Gpd-1 on Chromosome 4. This gene controlled the quantitative expression of XenCSA in lymphocytes.

Genetic studies of the Fv-1 locus in multiple recombinant inbred strains of mice revealed a close linkage between Fv-1 and Gpd-1; the estimated distance between these two genes was 0.6 cMs. Preliminary observations indicated that other loci affecting viral parameters, in addition to Fv-1, may also be linked to Gpd-1.

Two hair-deficient mouse mutants, hr/hr and nu^{str}/nu^{str} were shown to have increased expression of MuLV compared with their normal wild type (+/+ or m/+) controls.

The presence of a type C viral reverse transcriptase (RT, RDDP) and a 70S RNA was demonstrated in rabbit-lymphosarcoma tissue. The RDDP was partially inhibited by antisera to FeLV, RLV, pig and gibbon type C viruses. No virus was released from fibroblast cultures established from renal tumors induced in the rabbit by combined ethylurea and sodium nitrite treatment.

Ecotropic virogene expression was found to be the major, while not the sole, determinant of M-MSV resistance in AKR/J and AKXL-RI lines.

An apparently double-stranded RNA (~6000 daltons) from murine tumors, transformed cell lines, and early embryos was isolated and partially purified. This compound when injected i.v. into tumor-bearing mice caused complete or partial tumor regression, was strain-specific, antigenic, and blastogenic.

Results in attempts to suppress both genetically determined leukemias and chemically-induced (pulmonary tumors) by type-specific viral antisera (goat IgG) thus far required consideration of the xenotropic virus class as a possible cofactor in the lung carcinogenetic process.

Significance to Biomedical Research and the Program of the Institute: It is now possible, genetically, to ameliorate or eliminate cancer in a mouse

throughout its natural lifespan through breeding. By identifying the genes and loci involved in cancer susceptibility and the immunological and physical markers associated with the "high cancer" genes, it is now possible not only to identify the highly susceptible animal, but to study the biochemical, immunological and metabolic mechanisms controlled by these genes. The development of this information will help (1) elucidate markers to identify the cancer-prone individual; (2) determine how the genes operate in regulating the natural oncogene, with the goal of correcting deficiencies associated with the switching on of cancer cells in susceptibles.

Proposed Course: (1) Further development, characterization, and uses of recombinant inbred lines. (2) Study of the association of the viral group-specific antigen and complete virus with tumor development. (3) Determination of the relationship between genes controlling and influencing the expression of murine leukemia virus. (4) Study of the genetic control of endogenous MuLV expression. (5) Studies with chimeric mice. (6) Study of genetic control of viral-chemical carcinogenesis.

Date Contract Initiated: May 2, 1967

JOHNS HOPKINS UNIVERSITY (NO1-CP7-1027)

Title: Studies on the Molecular Basis of Viral Carcinogenesis

Contractor's Project Director: Dr. J. Thomas August

Project Officer (NCI): Dr. John Stephenson

Objectives: To isolate and purify structural viral proteins and virus-associated enzymes; characterize their biological, chemical, and immunological properties, and use them as probes for analysis of viral gene expression, in tumor tissues, as agents for possible immunological control of viral infection, and as reagents for detection of virus infection.

Major Findings: A number of major polypeptides of BALB/3T3 and NIH/3T3 cells were modified, or disappeared, after transformation. These included a characteristic triangle of polypeptides of about 60,000 daltons and pI 7.05-7.15; a pattern of six polypeptides plus some additional minor components of 55,000 to 65,000 daltons and pI 7.5-7.35; a cluster of proteins of 25,000 to 35,000 daltons and pI 5.8-6.2; and a cluster of low molecular weight, acidic polypeptides of 12,000 to 20,000 daltons including a prominent component of about 12,000 daltons and pI 6.4. Two polypeptides of 65,000 and 72,000 daltons were present in NIH/3T3 and transformed BALB/3T3 cells, but absent in normal BALB/3T3 cells.

The viral receptor on the cell membrane appeared to be a protein requiring lipid for either activity or integrity in the membrane. Receptor binding was shown to be a function of the protein moiety of the viral envelope glycoprotein. The binding reaction was Ca^{++} dependent.

Significance to Biomedical Research and the Program of the Institute: Specific protein products of virus genetic expression, purified and well characterized, provide the reagents to examine relationships between viruses in terms of gene products and to search for evidence of similar viral gene functions in human disease.

Secondly, these sub-virion proteins provide the means to determine whether effective immunological control of virally-induced disease could be instituted without complication by viral genetic material.

Proposed Course: This contract terminates August 31, 1978.

Date Contract Initiated: May 1, 1977.

LITTON BIONETICS, INC. (NO1-CP6-1029; successor to NO1-CP3-3211)

Title: Studies on Molecular Events Leading to Transformation by RNA Oncogenic Viruses

Contractor's Project Director: Dr. Marvin Reitz

Project Officer (NCI): Dr. Robert Gallo

Objectives: To characterize virus-like particles in human leukemic cells with respect to DNA polymerase and nucleic acids and characterize and purify viral reverse transcriptases from mammalian viruses, especially primate type C RNA tumor viruses.

Major Findings: It was found that some human DNA samples were capable of forming nuclease-resistant complexes with single-stranded nucleic acid probes from simian sarcoma virus (SiSV) and murine leukemia virus (MuLV). These complexes had a thermal stability and kinetics of formation consistent with the presence within the DNA samples of a set of DNA sequences related to but not identical with a portion of the viral genome. These sequences were found more frequently in leukemic than nonleukemic DNA samples, particularly with chronic myelogenous leukemia (CML). This type of distribution of sequences was not found with labeled probes from feline leukemia virus (FeLV), endogenous rat virus (V-NRK) and the Hall's Island strain of gibbon ape leukemia virus (GaLV-H). Some DNA samples, including a normal placental DNA and a CML cell line (K562), had a high level of these sequences, and were characterized in greater detail for specificty of these sequences.

A nonleukemic gibbon ape which had been exposed to a viremic animal and was persistently positive for GaLV antibody, but from whom no virus could be recovered, was examined for GaLV-related DNA sequences. The spleen, kidney and liver, but not the marrow or other tested tissues, appeared to contain an incomplete set of proviral sequences.

Fresh human hematopoietic cells were infected successfully with SiSV, GaLV, baboon endogenous virus (BaEV) and FeLV. In the case of the first two viruses, the cells were induced to grow independent of added growth factor, became

tumorigenic in nude mice and were able to form colonies in semisolid media. All these induced cells were EBNA-positive, suggesting that Epstein-Barr virus (EBV) and SiSV-GaLV were acting synergistically.

Significance to Biomedical Research and the Program of the Institute: The knowledge acquired is applied to the determination of the etiological relationship of viruses to leukemia in humans, to the development of diagnostic and prognostic modalities for human cancer, and ultimately, to the development of more effective control measures.

Proposed Course: This contract terminates June 30, 1978.

Date Contract Initiated: September 1, 1972

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP7-1007)

Title: Sequencing of the 3' End of RSV 35S RNA

Contractor's Project Director: Dr. Paul Zamecnik

Project Officer (NCI): Dr. Charles Sherr

Objectives: By means of oligonucleotide primer-initiated template copying of purified 35S RNA from both cloned RSV (Prague strain, subgroup C) and AMV (BAI strain A) with purified reverse transcriptase from AMV, the contractor will (1) determine the sequence of 100 or more nucleotides adjacent to the poly(A) sequence at the 3' terminus of oncornavirus 35S RNA and (2) determine the complete nucleotide sequence at the 5' end of AMV 35S RNA from the site of initiation by endogenous primer to the 5' terminus of the viral genome (approximately 100 nucleotides).

Major Findings: A sequence of nucleotides just internal to the poly(A) at the 3'end of AMV 35S RNA was determined. This run of nucleotides was very similar but not identical to the sequence existing in the comparable position in RSV 35S RNA.

A tridecamer deoxynucleotide complementary to a segment of the 21 nucleotide 3' and 5' reiterated sequence of RSV 35S RNA was synthesized, purified, and shown to provide the sequence planned. The tridecamer was added to chick embryo fibroblast tissue cultures infected with Rous sarcoma virus. Inhibition of virus production resulted. The inference emerged that the tridecamer and its counterpart with blocked 3'- and 5'-hydroxyl termini entered the chick fibroblast cell, hybridized with the terminal reiterated sequences at the 3' and 5' ends of the 35S RNA, and interfered with one or more steps involved in viral production and cell transformation. Likely sites of action

were (i) the circularization step of the proviral DNA intermediate, and (ii) the initiation of translation.

The tridecamer was also an efficient inhibitor of the translation of proteins specified by the viral RNA in the wheat embryo cell-free system. The inhibition specificity for oncornavirus RNA was greater than for rabbit reticulocyte mRNA or brome mosaic virus RNA. Other oligodeoxynucleotides of similar size had little or no specific effect on the RNA-directed translation. The tridecamer acted as a primer for the avian myeloblastosis virus DNA polymerase when Rous sarcoma virus heated 70S RNA was used as a template, offering evidence that it could hybridize to the RNA.

Significance to Biomedical Research and the Program of the Institute: The 3' terminus of 35S RNA of oncornaviruses almost certainly plays an important role in viral integration and replication. The endogenous primer molecules are now known to bind with 35S RNA subunits within 150 nucleotides of the 5' terminus; furthermore, preliminary evidence indicates that circularization of the viral genome occurs during an early stage of replication. Recent preliminary evidence suggests that some portion of the 3' terminal sequence is reiterated at the 5' end. These results suggest that one key to understanding the mechanism of replication of RNA tumor viruses involves determination of the nucleotide sequences at both the 5' terminus and the 3' region adjacent to the terminal poly(A) segment of the viral genome. Such sequence analysis may provide insight into the structural basis for formation of circular intermediates during replication. Ultimately, knowledge of the primary structure at both the 5' and 3' ends of the genome may indicate mechanisms for formation of a provirus structure and integration of the provirus into the host cell genome.

Proposed Course: The sites of inhibitory activity of the tridecamer deoxynucleotide sequences will be explored. *In vivo* tumor inhibitory studies will be pursued.

Date Contract Initiated: January 1, 1977

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP5-3562)

Title: Studies on the Leukemia Virus DNA Polymerase and Terminal Deoxynucleotidyl Transferase

Contractor's Project Director: Dr. David Baltimore

Project Officer (NCI): Dr. Edward Scolnick

Objectives: (1) Carry out a detailed analysis of DNA polymerases from RNA tumor virus particles and from normal and neoplastic cells. (2) Study the mechanism of double-stranded DNA synthesis by the avian myeloblastosis virus DNA polymerase and the functions of the subunits of the enzyme in infected cells and variation in DNA polymerases in various stages of cell growth.

Major Findings: Studies of reverse transcription in virions of MuLV showed that under defined conditions, very long molecules of complementary DNA could be made. These DNA molecules were infective in NIH/3T3 cells but actinomycin D blocked synthesis of infective molecules. The longest DNA made in the presence of actinomycin D lacked sequences from the 5'-end of the viral RNA as shown by heteroduplex analysis with 21S RNA that was presumably the mRNA for the glycoprotein of the virion. This RNA was shown to be a composite of 5'- and 3'-proximal sequences.

In other studies, it was shown that reverse transcriptase was made as a 180,000 molecular weight precursor that was cleaved in virions to the 85,000 molecular weight reverse transcriptase. The 180,000 protein had antigenicity of both gag and pol proteins and was made by a by-pass of UAG codon at the end of the gag gene. This codon could be suppressed in vitro with yeast amber suppressor tRNA but the in vivo mechanism remained unknown.

Studies of terminal deoxynucleotidyl transferase (TdT) continued to define it as a marker of maturing lymphoid cells probably of both the T and B lymphocyte series. In patients with blastic chronic myelogenous leukemia, occurrence of TdT was a very good indicator that remission induction with vincristine and prednisone would be successful.

Significance to Biomedical Research and the Program of the Institute: Fundamental studies on oncogenic virus replication are necessary to define the role of these viruses in malignant cell transformation. In vitro synthesis of infectious nucleic acid and in vitro translation of proteins allows first level mechanistic studies which can logically be expected to provide means for controlling the infections and/or transforming processes.

Proposed Course: Analysis of genetic control of viral replication will be analyzed. Mechanism of DNA proviral synthesis by reverse transcriptase and the function of RNase H will be explored. In vitro translation will be used to determine precursor sequences and cleavage sites.

Date Contract Initiated: May 1, 1971

MINNESOTA, UNIVERSITY OF (NO1-CP6-1055)

Title: Transcriptional Regulation of Eukaryotic Gene Sequences

Contractor's Project Director: Dr. Anthony J. Faras

Project Officer (NCI): Dr. Edward Scolnick

Objectives: (1) To delineate the control mechanisms involved in expression of RNA tumor virus genes in eukaryotic cells by performing studies on avian tumor virus genes in transformed and revertant vole cells in vitro; (2) to determine how the transformed phenotype is maintained; (3) to investigate the possible role of translation, polyadenylation, methylation, mRNA transpo

and processing in the post-transcriptional regulation of tumor virus gene expression.

Major Findings: Studies thus far indicated that no major restriction on either the transport of viral-specific RNA from the nucleus to the cytoplasm or the association of viral RNA with polyribosomes could be observed in revertant vole cells. Polyadenylic acid-containing viral RNA could also be identified in revertant cells in amounts similar to that found in transformed cells indicating that no differences in polyadenylation could be detected between the two phenotypes. Similar results were obtained with the sarcoma-specific RNA species. On the basis of genetic complexity studies and hybridization analyses with various sequence-specific cDNA probes, the majority of ASV genetic sequences in both transformed and revertant vole cells were investigated. To date no differences were detected in either the structure or size of viral RNA present in transformed and revertant vole cells.

Significance to Biomedical Research and the Program of the Institute: In an effort to gain an understanding of virus-induced oncogenesis, delineation of the control mechanisms involved in the regulation of RNA tumor virus genes, particularly those responsible for malignant transformation in eukaryotic cells, is essential. From the studies on transforming and revertant vole cells, conclusive evidence has been obtained indicating that the control of virus gene expression and virus-induced oncogenesis occurs at the post-transcriptional level in this cell system. Since virtually nothing is known regarding the mechanisms by which eukaryotic cells regulate the expression of their own genes, let alone oncornavirus genes at this level, investigation of transformed and revertant vole cells may elucidate the post-transcriptional factors that influence the genetic expression of transforming gene sequences in eukaryotic cells. Elucidation of the cellular and viral functions involved in these regulatory phenomena is likely to provide insight into the regulation of oncogenic viruses in eukaryotic cells and quite possibly contribute to an understanding of the mechanisms on oncogenesis in general. Furthermore, these studies may shed light on the mechanisms by which the exogenous infection of cells by RNA tumor viruses can, in some instances, result in the establishment of nonexpressed inherited viral genomes exemplified by the presence of endogenous viruses in normal cells.

Proposed Course: The contractor will further investigate the transformed/revertant vole cell system in an effort to elucidate the post-transcriptional factors that ultimately influence the genetic expression of transforming gene sequences in eukaryotic cells.

Date Contract Initiated: June 30, 1976

NEW ENGLAND MEDICAL CENTER (NO1-CP6-1046)

Title: Immunology of Murine Leukemia Virus Infection

Contractor's Project Director: Dr. Robert Schwartz

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Analyze human cancers for DNA sequences specific for RNA tumor viruses.

Major Findings: Considerable progress has been made in investigations on the relationship between pol Cm, a new polymerase previously detected in several cultured malignant human cancer lines, and human cancer. A procedure was developed whereby tumors and normal tissues were routinely screened for pol Cm activity. In addition, the total DNA polymerase level in unfractionated tissue extracts was determined by assaying under conditions optimal for DNA polymerase α. Some 46 human tissues were analyzed. Among tissue extracts calculated to contain sufficient overall DNA polymerase activity to detect potential pol Cm activity, 7 of 14 malignant and none of 11 normal or embryonic tissues were found to contain detectable DNA polymerase Cm. These data continued to support a strong association between pol Cm and human malignancy.

70S RNA was purified from 5 retroviruses [GaLV, WoSV(WoLV), BEV, FeLV, R-MLV] that had been tentatively associated with human cancer. Both [^{3}H]- and [^{32}P]-labeled probes were prepared, using the exogenous reaction with AMV-reverse transcriptase primed with calf thymus DNA or oligo(dT). These probes hybridized well. Although several different approaches were attempted, it has not been possible to prepare a WoSV sarc-specific probe.

A double-blind molecular epidemiology program to assess the role of retroviruses in human leukemia was begun. Procedures were developed for the extraction of "high molecular weight DNA" and RNA simultaneously from small samples of the same tissue. Using this procedure, DNA and RNA were isolated from 15 leukemia specimens recently received from the Virus Cancer Program. Isolation has also begun from over 100 small, histologically well characterized lymphomas.

Significance to Biomedical Research and the Program of the Institute: This research program is aimed at applying new information on viral carcinogenesis directly to the problems of human cancer.

Proposed Course: This contract will participate in a double-blind study to confirm the presence of primate oncornaviral information in human leukemic cells.

Date Contract Initiated: June 1, 1976

SALK INSTITUTE FOR BIOLOGICAL STUDIES (NO1-CP7-1008)

Title: Cellular Differentiation and Viral Oncogenesis

Contractor's Project Director: Dr. Hung Fan

Project Officer (NCI): Dr. Robert Goldberg

Project Officer (NCI): Dr. John Stephenson

Objectives: To determine (1) influence of the H-2 genetic region on the immune response of mice to murine leukemia virus (MuLV). (2) Correlation between genetically determined immunological responsiveness to MuLV and ability to eliminate MuLV. (3) Immunological responsiveness to MuLV administered across the tropism barrier. (4) Correlation between genetically determined immune response to MuLV and incidence of neoplasms. (5) Role of the complement system, if any, in elimination of MuLV. (6) Immunological and virologic correlates of the RgV-1 gene, the H-2 locus which specifies resistance or susceptibility to leukemia induced by several types of MuLV.

Major Findings: Mechanisms were identified that could eliminate or inhibit the replication of MuLV after infection had been established. These mechanisms were genetically controlled. Relevant loci mapped with the H-2 complex, but powerful resistance mechanisms were specified by a gene (or genes) located elsewhere. Resistance conferred by both H-2 and non-H-2 genes was dominant. Thymocytes were essential for expression of H-2 linked resistance to MuLV. Suppression of thymic function resulted in persistent, chronic, high-grade infection and ultimately neoplasia. It was not clear how thymocytes participated in resistance to MuLV. Differences in levels of anti-viral antibodies between susceptible and resistant mice were not decisive. An extensive search for cell-mediated immunity to MuLV in resistant mice was negative. The resistance mechanism was age-dependent and appeared within the first week of life; susceptible mice could not "switch on" this mechanism and were equally susceptible as adults and newborns to MuLV. This, combined with genetic mapping studies that pointed to the I-J region as the locus of the genetic mechanism, implicated a suppressor T-cell in this system.

Significance to Biomedical Research and the Program of the Institute: This program can help elucidate immunological and genetic markers predisposing to cancer, contributing to early detection of risk. Since cancer is at best difficult to treat and cure, early detection and prevention are of major importance.

Proposed Course: The mechanism of age dependence of immunoresistance to MuLV will be investigated. Resistance of C57L mice and their crosses to MuLV will be analyzed. Genetically-determined immune responses to purified viral proteins will be analyzed in detail. Correlations between expression of ecotropic and xenotropic viruses in immunosuppressed mice developing a reticulum cell sarcoma will be determined.

Date Contract Initiated: June 30, 1976

SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE (NO1-CP6-1049)

Title: Studies on the Mechanism of Oncogenesis by RNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Objectives: To study parameters controlling virus activation and expression during embryonal development and cellular differentiation.

Major Findings: The system used in these experiments was a line of BALB/c mice genetically transmitting the gene for Moloney murine leukemia virus (BALB/Mo mice). During this year a breeder colony of BALB/Mo mice homozygous for the genetically transmitted M-MuLV (MOV-1) gene was established. Somatic cell genetics and standard genetic experiments located the MOV-1 gene on chromosome No. 6. Permanent tissue culture lines also were established from mice heterozygous for the MOV-1 gene. These lines were virus negative, but were rapidly induced to express M-MuLV viral protein after induction with halogenated pyrimidines.

The expression of the MOV-1 gene in young animals was also studied. Two steps in amplification of the M-MuLV DNA were found: a preleukemic amplification, followed by a further amplification of M-MuLV DNA copies in the leukemic cells.

Several independently derived lines of fibroblasts productively infected with M-MuLV were prepared and characterized according to M-MuLV DNA copy number and virus production. Techniques were developed to study the integration sites of the M-MuLV DNA in these cell lines by restriction endonuclease digestion, "Southern blot-transfer" to nitrocellulose sheets, and hybridization with M-MuLV-specific cDNA probes.

Significance to Biomedical Research and to the Program of the Institute: Mounting evidence suggests that the state of cellular differentiation during retroviral infection is crucial to the outcome of the infection-transformation and/or chronic infection. This project centers on a unique situation in which tumor virus information inserted into the germ line of mice is monitored on the molecular level for expression during embryonal development.

Proposed Course: The contractor will monitor activation of MuLV at the transcriptional and translational levels in different tissues during embryonal development and after birth and study the molecular mechanisms involved in organ specificity of virus expression, infection, and transformation.

Date Contract Initiated: August 19, 1977

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP7-1018; successor to contract NO1-CP4-3375)

Title: Immunologic Study of Type C RNA Tumor Virus

Contractor's Project Director: Dr. Frank J. Dixon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This program is comprised of three integrated segments aimed at (1) identification of the various virion and non-virion-associated products of

endogenous type C virus genomes in mice, (2) quantitation of the spontaneous immune responses of mice to the products of their endogenous type C virus genomes and any immunopathologic consequences thereof, and (3) modification of expression of endogenous type C virus genomes by immunization with virus or purified virus antigen vaccines or by immunosuppression.

Major Findings: An extensive analysis of the tryptic peptides of the major virion structural proteins of viruses isolated from AKR mice was completed. Techniques were developed which allowed rapid analysis of all the virion proteins using only small amounts of virus. It was through use of this technology that the MCF viruses were conclusively shown to be env gene recombinants. Another host defense identified by this contract was the inactivation of RNA tumor viruses by complement mediated viral lysis, not requiring virion specific antibodies. A single, low molecular weight surface protein was identified as the virion macromolecule which binds early complement components, thus initiating the lytic cascade.

Significance to Biomedical Research and the Program of the Institute: This program is relevant to the goals of the National Cancer Institute in relation to determination of cause, early detection of risk, and eventual effective prevention of cancer. The etiological association between type C RNA viruses and cancer has been firmly establised in chickens, mice, hamsters, rats, and cats, and is strongly implicated in cows, gibbon apes, woolly monkeys and baboons as well. In addition, particles have been observed in human placentas in numbers suggesting that they are universally present. Expression of this apparently universal oncogenic potential, however, is dependent on the immunologic responses of the host. Although these immunologic controls are well recognized, the mechanisms are poorly understood. Characterization of the viruses, virus antigens and host cell responses will provide important insights into the etiological role of the viruses under varying immunologic conditions, and the means of detecting and interfering with their oncogenic properties through the use of viral vaccines.

Proposed Course: The contractor will continue ongoing studies, as follows: (1) Characterization of the products of endogenous type C viral genomes and their interaction with host immunologic defense mechanisms. (2) Analysis of the molecular nature of the viral genome products, and definition of the relationship of their production to host genetic or developmental events, including cancer. (3) Definition of the spontaneous immune responses of mice to their endogenous type C oncornaviruses and any associated immunopathologic events. (4) Attempts to manipulate natural production and control of endogenous type C viruses or non-virion-associated viral molecules to determine immunopathologic complications, effect on control of natural cancers and/or normal growth and development. (5) Definition of the potentially important direct neutralizing effect of primate complement on type C viruses and associated neoplastic events.

Date Contract Initiated: June 29, 1972

SIDNEY FARBER CANCER CENTER (NO1-CP7-1023; successor to NO1-CP5-3539)

Title: Attempt to Isolate Type C Virus from Cultured Human Leukemia Cells

Contractor's Project Director: Dr. David M. Livingston

Project Officer (NCI): Dr. George Todaro

Objectives: To attempt to isolate an intact type C virus from freshly-cultured human leukemia cells, either by finding such cells which spontaneously produce virus, or by inducing virus production by physical, chemical or immunological means.

Major Findings: The technique of immunoaffinity chromatography was applied to the development of lymphoid (and myeloid) cell lines of leukemic origin permitting a great reduction in the population of EBV-transformed B-cell derivatives present in initial cell stocks. The technique permitted the establishment of one of the first 'null' leukemia continuous cell lines (LAZ 221). It was free of surface IgG and was also EBNA negative. A human T-cell line was also developed (LAZ 191) bearing newly recognized surface antigens which were leukemia specific.

A number of 'leukemic' cell lines and viably frozen cell packs were cocultivated with a variety of xeno- and homogeneic cell lines so far without any success in type C viral isolation. A series of 18 DNA's from fresh or frozen human leukemic cell packs (marrow and/or peripheral blood) were screened for the existence of sequences homologous to the woolly type C virus. No definitively positive results were identified thus far.

The cystine auxotrophy, cystathionase depletion (CYS^-, CSE^-) phenomenon was shown to be a property of fresh leukemic cells from patients with ALL, AUL, and AML. It could be grossly correlated, in initial studies, with the number of leukemic cells in the bone marrow.

Murine liver cystathionase was purified to homogeneity. An antibody was raised against it; and the protein, once iodinated, could be specifically immunoprecipitated. Existing data suggests that the CYS^-, CSE^- phenotype was a result of the reduction in cellular content of active cystathionase protein.

Significance to Biomedical Research and the Program of the Institute: The successful isolation of a type C virus from human leukemia cells would permit the development of reagents to determine whether such viruses are natural etiological agents of this human cancer. For example, the ^3H-DNA product of the endogenous reverse transcriptase reaction of a human type C virus would be the ideal probe for the investigation of the partial genetic expression of such viruses in nonproductive, normal and malignant human cells. Specific proteins of such a particle would be superb tools for the development of analytical systems designed to detect type C viral proteins in nonproductive human cells. The identification of a viral-specific transformation protein in transformed, but not normal cell, would be the strongest evidence that endogenous type C virus is involved in the etiology and pathogenesis of human neoplasia.

Proposed Course: This contract will terminate September 30, 1978

Date Contract Initiated: April 24, 1974

SIDNEY FARBER CANCER INSTITUTE (NO1-CP7-1051)

Title: In Vitro Study of the Interrelationships Between BALB:virus-1 and Myeloid Cell Differentiation in NIH/Swiss Mice

Contractor's Project Director: Dr. Joel S. Greenberger

Project Officer (NCI): Dr. John R. Stephenson

Objectives: To define a system for viral transformation of bone marrow myeloid cells in vivo and in vitro, to delineate the stages of differentiation of myeloid cells which will support viral infection and transformation, and to define the characteristics of the transformed state.

Major Findings: A biological effect of BALB:virus-1 was demonstrated in vitro: induction of proliferation of purified myeloid shifted NIH/Swiss adult mouse granulocyte-macrophage (GM) progenitor cells in the absence of added colony stimulating factor. Using a 10-step discontinuous Ficoll-Hypaque gradient, GM-progenitor cells were concentrated 4-10 fold in the top 4 fractions with more differentiated cells in lower fractions. Each gradient fraction was infected in vitro with BALB:virus-1 (KiMSV), Rauscher-MuLV, or R-MuLV (KiMSV). Cells were transferred to colony assay in 0.8% methycellulose-containing medium. Colonies were scored alone or in the presence of CSA (L-cell source) (CFUc), added erythropoietin, or LPS and 2-mercaptoethanol. High frequency colony formation in the absence of CSA was detected in light density fractions infected with the host range of the inoculated virus, and were granulocyte-macrophage in morphology, histochemistry, and surface membrane markers. Virus-infected GM colonies grown in CSA were larger and contained more immature cells per colony than uninfected controls. These data indicated that BALB:virus-1 as well as other RNA type C viruses could grow in GM-progenitor cells. Thus, the infrequency of granulocytic leukemias in mice cannot be attributed to a block in infection and/or growth of virus in these particular target cells.

Using a continuous bone marrow culture system, marrow from NIH/Swiss mice was maintained for 4 weeks, then recharged with a second adult mouse femur marrow and BALB:virus-1, Rauscher-MuLV or KiMSV pseudotype viruses. While KiMSV infection of bone marrow cultures caused disappearance of proliferating colony-forming cells in the nonadherent fraction with replacement by malignant macrophage-like cells which induced monomyelogenous leukemia in newborn mice; cultures infected with BALB:virus-1 or Rauscher MuLV produced more nonadherent cells compared to uninfected cultures, and cells removed were shifted to greater numbers with immature morphology. There was an increase in total number of CFUc, compared to uninfected cultures, and larger numbers of independent colony forming cells. Thus, BALB:virus-1 as well as Rauscher-MuLV demonstrated early biologic effects in long-term bone marrow culture.

To enrich for granulocyte-macrophage progenitor cells, a technique of immunoaffinity chromatography for cell-surface-lysozyme was designed. Technology for this method was worked out with human lysozyme. Immunization of goats with human lysozyme produced high titered specific antisera by 2 months. Monospecific antihuman lysozyme was eluted by chromatography against human lysozyme bound to sepharose beads or $F(ab')_2$ fraction prepared by pepsin digest and bound to sephadex G200. Peripheral blood or bone marrow cells were passed over the columns and adherent vs. nonadherent cell fractions quantitated by morphology and histochemistry. Results demonstrated >80% of the adherent cells to be monocytes or, with marrow, GM cells.

Significance to Biomedical Research and the Program of the Institute: This project represents one of the few systems available for in vivo and in vitro induction of myelogenous leukemia. Exploitation of this system will enable the contractor to make significant contributions to definition of the mechanism of transformation of the myelogenous hematopoietic system.

Proposed Course: The contractor will develop systems for separation of subpopulations of the bone marrow hematopoietic compartment. The myeloid cells will be used to study BALB:1 viral infection and parameters of transformation.

Date Contract Initiated: August 15, 1977

SIDNEY FARBER CANCER INSTITUTE (NO1-CP7-1057)

Title: Studies on the Integration Sites of Oncogenic Viruses

Contractor's Project Director: Dr. William Haseltine

Project Officer (NCI): Dr. Takis S. Papas

Objectives: To characterize the sites of integration on Moloney murine leukemia virus (MoMuLV) and Rous sarcoma virus (RSV) and host cell genomes; to examine the integration of EBV genome on the human B-lymphocyte genome.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance for Biomedical Research and the Program of the Institute: The integration site of viral genomes on the host cell genome may have an effect on initiating the malignant state. This contract will yield significant information regarding the integration sites of MoMuLV, RSV and EBV.

Proposed Course: The 3' and 5' terminal nucleotide sequences of the RNA genomes of MoMuLV and RSV grown on different host cells will be determined and compared to determine the origin of the transforming sequences. The status of the EBV genome in infected human B-lymphocytes will be determined.

Date Contract Initiated: September 1, 1977

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1003)

Title: Influence of Virus-Related Genes on Susceptibility to Cancer

Contractor's Project Director: Dr. Edward A. Boyse

Project Officer (NCI): Dr. Stephen O'Brien

Objectives: (1) The breeding, maintenance, and supply of congenic strains of mice which differ from their partner strains in expression of genes concerned with production or control of leukemia virus, and (2) testing these paired strains for their susceptibility to spontaneous and induced malignancy.

Major Findings: The funds made available to this contract were devoted to: (a) the continued production of congenic mouse strains that carry discriminative alleles of genes of special relevance to type C viral oncology, on background genotypes of established inbred strains; and (b) help meet the cost of distributing these mice, when they became available, to all investigators who requested them.

The main loci concerned were Gv-1, Gv-2, Fv-1, Akvp and the MHC complex. In some instances, two congenic strains were prepared, in which the differential alleles of a gene distinguishing two unrelated inbred strains were exchanged. These 'quartets', consisting of two inbred strains and the respective congenic strains, permitted the breeding of pairs of congenic F_1 hybrids, which offer several advantages and additional models, in comparison with conventional congenic pairs. These congenic hybrids were also made available to investigators who asked for them.

A remarkable example of the value of 'quartets' was the discovery of an autoimmune disease that affects B6/Gix(+) x 129 hybrids, but neither parent strain, nor the congenic B6 x 129/Gix(-) hybrid stock.

Significance for Biomedical Research and the Program of the Institute: Genetic control of susceptibility to spontaneous and viral-induced leukemia in mice has been well documented. However, the mechanism of control by the several loci involved has only recently received attention. The present study has been designed to assign specific functions to each controlling genetic allele. Identification of genetic control mechanisms in murine strains should form the groundwork for identifying similar controlling factors in other species, including man.

Proposed Course: "Quartets" of four strains, representing two base strains and two corresponding congenic strains in which the differentiating alleles have been switched both ways, will be developed and supplied to all qualified interested investigators. Seven quartets are planned covering the H-2, Fv-1, PC, TL and G_{Ix} loci. The influence of each of the alleles on expression of

virus, host response to viral products, and occurrence of leukemia will be investigated by monitoring virological, immunological and pathological parameters during the life span of these strains.

Making use of congenic stocks already established, selected 'double' congenic strains will be made in which alleles of two loci will be substituted on a genotypic background common to that on which each allele was individually isolated in the first place. This will permit assessment of the joint action of alleles of two genes, as compared with either alone, on virus-related charcteristics and on susceptibility to neoplasia and other diseases associated with type C virus.

Date Contract Initiated: December 15, 1976

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1053)

Title: Study of Interrelationship Between MuLV Infection and Myeloid Cell Differentiation

Contractor's Project Director: Dr. Malcolm A.S. Moore

Project Officer (NCI): Dr. David H. Troxler

Objectives: To establish long term steady state cultures of mouse bone marrow and to monitor such cultures infected with MuLV for evidence of transformation; to correlate changes during transformation and infection.

Major Findings: Replication of cloned Friend ecotropic helper virus (F-MuLV) or of SFFV with F-MuLV was obtained in continuous murine bone marrow culture. In vitro transformation of hemopoietic stem cells (CFU-s) and progenitor cells (CFU-c) was detected after 8 weeks in cultures infected with F-MuLV alone. Transformation was associated with an increase in production of CFU-s which retained pluripotentiality and capacity to reconstitute lethally irradiated mice. The transformed CFU-s proliferated in agar culture and replicated in simple suspension culture in the absence of an adherent microenvironment. In vivo, transformed CFU-s exhibited extensive if not unlimited self-renewal capacity following serial passage in irradiated mice. Transformation of CFU-c altered their maturation pathway and endowed this progenitor population with a stem cell capacity as detected by continuous generation of CFU-c in suspension culture in the presence of colony stimulating factor. These properties appeared to represent a preleukemic phenotype. Spontaneous leukemic transformation was noted in certain allogeneic bone marrow culture combinations and in coculture of CBA bone marrow and fetal liver. Kirsten-MSV infection of marrow cultures resulted in transformation of the macrophage component of the adherent marrow microenvironment with associated loss of its capacity to sustain stem cell replication and continuous hematopoiesis. Abelson virus infection of marrow cultures produced a delayed transformation associated with blast cell conversion, marked increase in cell production and appearance of agar cloning capacity in the presence of 2-ME. Transformation of marrow

cultures was noted even when addition of the virus was delayed for up to 4 weeks indicating that the target cell for transformation persisted and probably replicated in vitro for this length of time.

Significance to Biomedical Research and to the Program of the Institute: Careful analysis during in vitro transformation of virological, functional, morphological, biochemical, and genetic parameters will yield important information regarding fundamental changes during initiation of the malignant state of hematopoietic cells.

Proposed Course: Long term steady state cultures of mouse bone marrow will be established, infected with Friend leukemia virus, and monitored for evidence of transformation and virus infection.

Date Contract Initiated: August 15, 1977

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1059)

Title: Structure and Distribution of Integrated Sequences of Feline Oncornaviruses

Contractor's Project Director: Dr. Wolf Prensky

Project Officer (NCI): Dr. Stephen J. O'Brien

Objectives: To determine the integration sites of feline leukemia virus (FeLV) and endogenous virus (RD-114) in cat cells by somatic cell hybridization and in situ hybridization techniques.

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: It is possible that the site of FeLV integration may be responsible for the consequences of infection. Interaction with endogenous RD-114-related genetic information may also play a role. This project will use the various types of tumors of the cat as input material for studying the integration sites of FeLV and RD-114 at the chromosomal level to determine whether the nature of the integration site(s) play any role in oncogenicity or expression of the viral genome.

Proposed Course: The contractor will establish, by cloning, a number of mouse cell lines containing cat chromosomes, and test for presence of feline proviral sequences by in situ hybridization. The integration site will be mapped in relation to other markers of the cat genome. The synteny of exogenous FeLV sequences with specific chromosomes and marker genes will be tested. Hybrids of mouse and Asian cat cells infected with FeLV will be prepared and tested for integration sites.

Date Contract Initiated: September 28, 1977

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP5-3518)

Title: Conditional Lethal Mutants of RNA Tumor Viruses

Contractor's Project Director: Dr. Peter K. Vogt

Project Officer (NCI): Dr. John R. Stephenson

Objectives: (1) To isolate temperature-sensitive (ts) mutants in genes coding for virion proteins of avian sarcoma viruses. (2) To isolate ts transformation mutants from avian RNA tumor viruses which differ in origin and pathogenicity from RSV or B77. (3) To isolate nonconditional deletion mutants of avian RNA tumor viruses.

Major Findings: A series of ts src mutants from the Schmidt-Ruppin strain of Rous sarcoma virus were isolated to be used in mapping studies with deletion mutants of the same virus strain which have lost part of the src gene. From the Prague strain of Rous sarcoma virus new partial src deletions were isolated also to be used in mapping studies with ts src mutants of the same virus strain.

Avian carcinoma virus MH2 was studied for temperature sensitivity in the maintenance of transformation. The standard stocks of this virus showed such temperature sensitivity, but it was not known whether this was a viral or cellular property.

Temperature-sensitive mutants were isolated of the Carr sarcoma virus PRC-2.

Passage of partial src deletions in certain cell cultures and in the animal led to the acquisition of transforming activity for fibroblasts.

Significance to Biomedical Research and the Program of the Institute: The work performed by this contractor has provided valuable genetic tools for study of oncornavirus gene functions in replication and transformation and location on the viral genome. Future production of additional mutants from all segments of the genetic map will provide valuable tools for isolation and characterization of those gene functions relating specifically to potential viral carcinogenesis.

Proposed Course: This contract will continue to isolate new mutants of avian RNA tumor viruses.

Date Contract Initiated: October 15, 1971

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP4-3214)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. R. L. Heberling

Project Officer (NCI): Dr. Alfred Hellman

Objectives: To study the viral flora in placentas and embryos of primates with emphasis on type C viruses.

Major Findings: The biological properties of the endogenous primate retroviruses of the baboon (BaEV) and squirrel monkey (SMRV) were investigated. An in-depth study of the infectious process of BaEV following in utero or neonatal inoculation of the beagle dog was undertaken. Persistent infection and immunological tolerance for BaEV antigens were more likely to occur with in utero inoculation. After the 50th day of gestation, the development of a functional humoral and cell-mediated immunity suppressed the expression of infectious virus. Immunologic responses detected included SN, RIP, and anti-RDDP antibodies, and cytotoxic, lymphocyte blastogenesis, and MIF responses. Nucleic acid hybridization studies indicated that in tissues where infectious BaEV persisted, it was likely that only a few cells were involved. Beagles inoculated at birth with KiMSV(BaEV) a transforming virus, developed fibrosarcomas but immunologic responses suppressed virus expression and tumor progression in these instances as well. The pathologic response to KiMSV(BaEV) solid tumors or subcutaneous hemorrhaging with the accumulation of serosanguinous fluid, was a function of the infectious dose. Chronic DES treatment of BaEV inoculated dogs was not oncogenic.

In a study of the autogenous immunity of baboons to BaEV in relation to age, sex, and pregnancy, it was found that, as nonspecific responsiveness to mitogens decreased with age, humoral and cell-mediated responses to BaEV increases. In addition, responsiveness to BaEV also increased during pregnancy, accompanied by a suppression of mitogen responsiveness. The latter appeared to be related to a factor(s) found in the sera of pregnant baboons.

Hyperimmunization of baboons with Herpesvirus saimiri increased cell-mediated and humoral immune responsiveness to BaEV. Preliminary studies on the in vitro induction of BaEV from baboon lymphoid and fibroblastic cell cultures with mitogens and/or UV inactivated H. hominis were not consistently positive. Several isolates were made from lymphoid cells following mitogen stimulation.

Significance to Biomedical Research and the Program of the Institute: Evidence was obtained for the existence of vertically transmitted type C viruses in "normal" primate tissue. Further study is required to determine whether these agents have any direct relationship to the development of malignancy in any primate.

Proposed Course: This contract terminates September 15, 1978.

Date Contract Initiated: June 3, 1971

STANFORD UNIVERSITY (NO1-CP4-3228)

Title: Virologic, Biologic and Immunologic Characterization of Hodgkin's Disease and Other Human Malignant Lymphomas

Contractor's Project Director: Dr. Henry Kaplan

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To collect Hodgkin's disease (HD) tissue and serological samples for study. To cultivate human lymphoma tissue in vitro and characterize the established cell lines and clones derived from them. To investigate cultures for presence of virus, and characterize any viruses detected. To characterize immunologically the cells in relation to the host autoimmune responses.

Major Findings: Studies of the type C RNA virus produced by the SU-DHL-1 human histiocytic lymphoma cell line continues. Its reverse transcriptase was purified and partially characterized; antibodies prepared against it also partially inhibited the reverse transcriptase of SSV-1 and GaLV, and to a lesser extent, BaEV, RD-114, and MuLV. The virus was infectious for selected B-lymphocytic human cell lines, which became stable producers; human and other fibroblastic tumor cell lines were non-permissive. After cocultivation with killed DHL-1 cells or infection with free virus, normal human peripheral blood mononuclear cells consistently exhibited atypical proliferative responses in vitro, followed by secondary cytopathic changes; sustained transformation was not yet observed.

The spontaneous onset of sustained virus production, as revealed by reverse transcriptase activity in supernatants and by positive XC cell assays, was observed in several of our other human lymphoma cell lines.

Several new cell lines were established. Surface marker, cytochemical, and functional studies of 10 DHL cell lines revealed that 2 (DHL-1 and -2) were true histiocytic, 6 are B-lymphocytic, and 2 were 'null' cell in origin. Cytogenetic studies revealed the 8q-/14q+ translocation in all 3 of the American Burkitt's lymphoma lines; of these, AmB-1 was negative, whereas AmB-2 and -3 were positive for the EBV genome by the EBNA test. Abnormalities of Chromosome 14 were also seen in several of the B-lymphocytic DHL lines, whereas the true histiocytic and one null cell line had a 6q- marker.

Very encouraging preliminary results were obtained with Teflon film substrates for in vitro cultivation of Sternberg-Reed and Hodgkin giant cells from involved tissues of patients with Hodgkin's disease. Two such cultures were successfully transplanted to the nude mouse brain and re-established in secondary culture from the mouse.

Progress was made in characterizing the E-rosette inhibitory substance previously detected in the sera and spleens of patients with Hodgkin's disease. Several other in vitro tests of cell-mediated immunity were explored to ascertain their correlation with the impaired delayed hypersensitivity responses of these patients.

Significance to Biomedical Research and the Program of the Institute: Hodgkin's disease has the attributes of an infectious process. This project focuses available technology to detect evidence for a virus etiology in this malignancy. These studies may provide new information applicable to diagnosis, prognosis and treatment of Hodgkin's disease.

Proposed Course: The project will continue studies to further characterize the type C virus from the SU-DHL-1 cell line, to determine its oncogenic properties, and to investigate its relationship to HD.

Attempts will be made to convert HD cell cultures to permanent monotypic cell lines, using in vitro-in vivo selection techniques. It is felt that such monotypic cell lines could be used to induce heterologous antibodies, possibly of use for determining tumor-specific antigens shared with other HD cells. Attempts will be made to infect EBNA-negative HD cells with EBV by cocultivation. Once immediate cell cultivation goals have been achieved, attention will be turned to establishing cell cultures of other types of human malignant lymphomas which have not as yet been successfully cultivated in vitro.

Attempts will be made to purify the low-density lipoprotein-C-reactive protein complex which exhibits rosette-inhibitory reactivity to determine in which moiety of the protein complex the activity resides. Investigation will be carried out on this factor for effects on other types of immunologic responses in vitro. It is hoped that these studies will have relevance to the impairment of cell-mediated immunity observed in HD patients.

Date Contract Initiated: April 1, 1974

STANFORD UNIVERSITY (NO1-CP7-1052)

Title: In Vitro Study of the Interrelationship Between RadLV Infection and Lymphocyte Differentiation in C57B1/KA Mice

Contractor's Project Director: Dr. Henry S. Kaplan

Project Officer (NCI): Dr. Steven R. Tronick

Objectives: To study the differentiation-specific restriction system of RadLV lymphocyte infection in C57B1/KA mice.

Major Findings: Conditions for the in vitro cultivation of mouse thymus epithelial cells were explored. It was found that conditions for primary trypsinization are critical for establishment of epithelial cultures from thymus tissue. The doubling time of the epithelial cells in vitro was in excess of 96 hours. Epithelial cell cultures were used as feeders for thymocyte and bone marrow cell cultures.

Preliminary virus infectivity experiments indicated that very few thymocytes could sustain active replication of RadLV for more than four days.

Methods for the separation and enrichment of thymus and bone marrow cultures were investigated.

Preliminary evidence was obtained for the functional maturation of prothymocytes to T-lymphocytes during cocoltivation of bone marrow cells with thymus epithelial cells.

The spontaneous incorporation of tritiated thymidine into thymocytes preincubated on epithelial cell cultures was higher than in controls, indicating that more thymocytes were in S phase after incubation on epithelial cell cultures. Such cultivation also increased the responsiveness of normal thymocytes to PHA and con A mitogen treatments probably due to a maturation event.

Significance to Biomedical Research and the Program of the Institute: The patterns of replication and oncogenic activity of the MuLV endogenous virus RadLV in C57B1/KA mice are jointly determined by the virus and by the state of differentiation of the host cells in which it is induced by radiation or which it infects secondarily. This study will investigate this system at the molecular level.

Proposed Course: Subpopulations of candidate target cells from C57B1/KA mice will be prepared, characterized, and analyzed for the interaction of virus expression and stage of differentiation, during the transformation process.

Date Contract Initiated: August 15, 1977

TEXAS, UNIVERSITY OF (NO1-CP6-1017)

Title: Biosynthesis of Oncorna Proteins in Mouse and Human Cells

Contractor's Project Director: Dr. Ralph B. Arlinghaus

Project Officer (NCI): Dr. Takis S. Papas

Objectives: To study the synthesis and processing of murine leukemia virus proteins in mouse and human cells.

Major Findings: Strong evidence was obtained that Rauscher murine leukemia virus (R-MuLV) reverse transcriptase (RT) was made by synthesis and processing of a 200,000 molecular weight polyprotein containing *gag* and *pol* sequences.

Intermediate *pol* related precursors of 145,000 ($Pr145^{pol}$), 135,000 ($Pr135^{pol}$) and 125,000 ($Pr125^{pol}$) were shown to be present in R-MuLV infected cells. A 80,000 molecular weight *pol* protein was found in infected cells. It was similar in size and properties to mature virion RT.

Pulse-chase kinetics and peptide mapping studies indicated that RT was made in the following way:

$$Pr200^{gag\text{-}pol} \rightarrow Pr145^{pol} \rightarrow Pr135^{pol} \rightarrow Pr125^{pol} \rightarrow Pr80^{pol}$$

Intracellular $Pr80^{pol}$ might be further modified to yield virion $p80^{pol}$.

Intracellular Moloney murine leukemia virus (Mo-MuLV) precursors were characterized by immunoprecipitation and SDS-PAGE. They were similar to those previously described for R-MuLV except for some minor size differences in the env and gag precursors.

A 63,000 and a 95,000 molecular weight gag -related polypeptides unique to Moloney murine sarcoma virus (Mo-MuSV) infected cells were detected in Mo-MuSV transformed cells. Studies with a ts mutant of Mo-MuSV (transforming mutant) were begun.

The order of proteins within the gag precursor $Pr65^{gag}$ was studied by comparing peptide maps of premature termination products produced in a cell-free protein synthesis system. The tentative order was:

$_2$HN-p15-p12-p30-p10-COOH

Significance to Biomedical Research and the Program of the Institute: Oncornaviruses cause some types of cancer in many different animal species. By inference, one may assume that a viral etiology also exists for some types of cancer in man. In animal cells, viral information is often incomplete or incompletely expressed so that virus particles may not be assembled in some tissues. Similarly, in some human cancers, oncogenic viral information may be only partially expressed, accounting for the difficulty in establishing evidence for a viral etiology of human cancer. An immunological probe could serve as a basis to develop a clinical assay for oncogenic viral gene products in human tissues. Furthermore, the information obtained in this study is expected to further the knowledge of the relationship of these viral-specific gene products in the transformation process.

Proposed Course: The overall ovjective of this project is to prepare a suitable immunological probe to detect oncornavirus proteins in human cells. The specific aims include the study of the synthesis and processing of the Rauscher murine leukemia virus proteins, produced in mouse and human cells by the use of antisera prepared against components of disrupted virus particles. The antisera will be used as probes to compare, contrast and characterize the intracellular, viral-specific polypeptides synthesized in virus-infected human and mouse cells. Antisera prepared against disrupted human cell-adapted Rauscher virus, candidate human tumor viruses, and simian sarcoma virus will be used as immunological probes to detect oncornavirus gene products in human cells.

Date Contract Initiated: January 1, 1976

VIRGINIA, UNIVERSITY OF (NO1-CP7-1056)

Title: Physical and Biochemical Mapping of the Integration Sites of Avian Sarcoma Virus

Contractor's Project Director: Dr. J. T. Parsons

Project Officer (NCI): Dr. Gordon Hager

Objectives: To utilize DNA restriction endonucleases to identify and quantitate the site(s) of integration of avian sarcoma virus (ASV) provirus DNA in duck cells and mouse cells transformed by ASV.

Major Findings: These studies were undertaken to identify biochemically and quantitate the site(s) of integration of avian sarcoma virus (ASV) in duck and mouse cells. DNA purified from either ASV-transformed duck or mouse cells or subcellular fractions thereof were digested to completion with restriction endonucleases of known specificity. The cellular DNA restriction fragments were separated by agarose gel electrophoresis, transferred to cellulose nitrate filter paper and virus-specific restriction fragments located by hybridization with ^{32}p complementary viral DNA. A preliminary restriction map of the ASV genome was established by analysis of the virus-specific DNA fragments generated by restriction endonuclease cleavage of unintegrated forms of viral DNA (linear and covalently closed circular DNA). Experiments in which the restriction endonuclease pattern of viral DNA fragments of unintegrated DNA were compared with that of DNA purified from duck cells fully transformed by mass infection with B_{77}-ASV (integrated viral DNA) suggested that ASV DNA sequences were integrated at numerous sites on the duck genome. In a parallel study, two cell lines, B_{77}-ASV transformed 3T3 cells and Schmidt-Ruppin (SR)-transformed 3T3 cells, were examined. From each cell line a viral restriction fragment was identified which contained viral DNA sequences from the 3' end of the viral genome as well as cellular DNA sequences. Analysis of terminal restriction fragments of two cell lines generated by different restriction enzymes suggested that the viral genome was not integrated at an identical site on the cell chromosome.

Significance to Biomedical Research and the Program of the Institute: Infection of avian cells by non-defective avian sarcoma viruses leads to cellular transformation and virus replication. Similarly, certain mammalian cells infected with avian sarcoma viruses are transformed although the frequency of this event is considerably lower. A pivotal step in the establishment of transformation and in virus replication is the conversion of the infecting avian sarcoma virus (ASV) RNA genome into a DNA provirus and the integration of this provirus DNA into the host chromosome. The requirement for provirus DNA integration appears to be a specific and obligatory step for the expression of viral gene functions. The steps by which the RNA genome is converted to a DNA provirus and subsequently integrated in the host chromosome remain essentially unresolved.

Recent advances in DNA restriction enzyme technology and DNA fragment separation techniques now provide methods by which viral DNA fragments can be selectively separated from a large part of the host cell DNA. This study will consider the problem of biochemical identification and purification of integrated oncogenic viral sequences in cellular DNA. ASV-transformed mammalian cells and ASV-transformed avian cells present an ideal model system in which to initiate such studies on the integrated viral genome.

Proposed Course: These studies are designed to look at several apsects of ASV provirus integration and expression.

DNA restriction endonucleases will be utilized to identify and quantitate the site(s) of integration of ASV provirus DNA in duck cells and mouse cells transformed by avian sarcoma viruses. Cleavage maps will be generated using a number of enzymes. cDNA probes of limited complexity will be used to examine the sequence distribution within the different DNA fragments.

Date Contract Initiated: September 1, 1977

YALE UNIVERSITY (NO1-CP7-1060)

Title: Studies of Integration Sites of Sarc Gene of Moloney Murine Leukemia Virus

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To determine the site(s) of integration of the murine src gene in murine cells using somatic cell hybridization techniques.

Major Findings: Two different studies were pursued on the integration into mammalian genomes of viral gene sequences. Sequences in question were those of herpes simplex virus 1 in transformed mouse tissue cultured cells and endogenous sarc gene sequences in the normal genomes of inbred strains of mice.

Transfection experiments with HSV-1 in which thymidine kinase (TK) was used as a selectable prototrophic marker yielded two classes of transformed cells: stable and unstable. The hypothesis was tested that the stability phenotype could be explained by virus genome integration into a recipient cell chromosome. The method of analysis was by means of somatic cell genetics. A series of microcell hybrids were isolated between a TK^- Chinese hamster cell line and a transformed mouse cell line expression TK encoded by HSV-1. Several of the hybrid lines were shown to contain a single murine chromosome and these expressed only the viral TK. Karyotypic analysis of these hybrids and to TK^- derivatives generated by BUDR counter selection revealed that the viral TK^+ phenotype was correlated with the presence of the terminal portion of the long arm of the specific murine chromosome. The results were consistent with the hypothesis that the viral tk gene was covalently integrated into this chromosome region which itself did not appear to carry the endogenous murine tk locus. These findings showed that somatic cell genetics could be used to localize viral integration sites in host chromosomes with high resolution.

In other studies mouse/human hybrids which segregate normal mouse chromosomes were examined. These mouse chromosomes were derived from primary mouse cells explanted from mouse embryos. Approximately 20 individual hybrid lines were produced by various hybridization methods. These hybrids in aggregate represented a broad spectrum of individual mouse chromosomes. To date, a

series of chromosomes were eliminated as carrying the corresponding sarc sequences. One of the hybrid clones gave a positive reaction.

Significance to Biomedical Research and to the Program of the Institute: The occurrence of src virogene sequences in murine cells suggests a vertical line of transmission. This study will locate these sequences on specific chromosomes and lead to determination of control mechanisms of viral replication and transformation.

Proposed Course: The contractor will construct mouse-human somatic cell hybrids and will use these hybrids to locate integrated proviral sequences in the cellular genome of inbred strains of mice.

Date Contract Initiated: September 28, 1977

C. 2. OFFICE OF PROGRAM RESOURCES AND LOGISTICS

The Office of Program Resources and Logistics, Viral Oncology Program, is responsible for planning, initiating, and maintaining a coordinated program to anticipate and meet the needs of the intramural and the extramural Viral Oncology Program for research resources and logistical support. This coordinated program includes the initiation, development, maintenance, and management of resources contracts, responsibility for the day-to-day general management and direction of all resources distribution, and the development and maintenance of a highly sophisticated computerized inventory and management information system for the research resources acquired and utilized by the overall Viral Oncology Program. The Office was established by NCI in 1972 to centralize the scientific administration and management of research resources and logistical functions and to unify these activities for the Viral Oncology Program.

Laboratory cancer research investigations carried out in the Viral Oncology Program depend on the availability of viruses, viral reagents, antisera, animals, and clinical and laboratory materials of quantity, optimal purity, viability, and potency. Research studies in an integrated program of international scope, as encompassed in the collaborative extramural research program, make more meaningful and rapid progress when adequate quantities of such standardized reagents, cell cultures, and test animals are available. The Office of Program Resources and Logistics provides these research materials and other supportive activities through contract operations representing six general areas of activities. These include:

Activities directed toward production and characterization of purified viruses, viral reagents, and appropriate antisera.

Activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimen material.

Activities concerned with animal resources, including production of pathogen-free and germ-free species of animals, breeding of primates, maintenance of animal colonies, and containment-type animal holding facilities.

Activities directed toward the provision of specialized testing services for the examination of experimental materials.

Activities concerned with providing contract services for the biohazard safety program.

Activities directed toward providing laboratory facilities and services for direct support of NCI intramural research programs.

The overall requirement for research resources and the number and extent of requests received frequently exceed the availability of Program resources. This is due, to some extent, to the high cost of producing or preparing certain scarce reagents, to fiscal considerations which limit the ability to prepare every potentially necessary item, and to a reluctance to prepare highly specialized and expensive materials which can be utilized by only a few laboratories. Because requests do frequently exceed availability, the distribution of resources is influenced by the relative value of specific research in relation to overall Program goals. These relative values are determined by the VOP Coordinating Committee, the chartered Scientific Review Committees (peer review), the OPR&L Advisory Group, and the VOP Laboratory, Branch, and Office Chiefs. The distribution of resources is generally patterned on the evaluations and recommendations of these groups. Periodic or one-time requests from collaborating investigators for modest quantities of readily available research materials are usually processed by the office staff. When requests are of unusual diversity or magnitude, or when repeated over a period of time come to represent significant quantities, a special review and evaluation mechanism is introduced. For contractors, final determinations are made after suitable discussions and consultations with the appropriate project officer and the extramural program staff. For grantees, determinations are made after similar consultations with the grant program administration.

The principal intramural advisory committee assisting OPR&L in carrying out its functions is the Program Resources and Logistics Advisory Group. The PR&L Advisory Group constitutes a standing intramural committee to provide support and make recommendations concerning resources matters, and to conduct appropriate reviews for specific contracts administrated by OPR&L. The Advisory Group is chaired by the Chief, OPR&L, and the membership includes scientist-managers representing the Collaborative Research Branch, VOP, a representative of the VOP Office of Biohazard Safety, and also representatives from the four major intramural Laboratories of the Viral Oncology Program. The current membership of the Group is listed in a previous section of this report.

During the year, OPR&L has coordinated the distribution of a wide variety of biological, chemical, and materiel resources to NCI intramural investigators and VOP participants. The amount of material processed has been extensive. To illustrate the scope of activity, during this period the purified and concentrated products from approximately 50,000 liters of tissue culture-grown viruses, propagated in over 30 different cell lines, were distributed in over 1,100 shipments to over 400 participating laboratories throughout the world. Additionally, over 250 grams of a single virus not propagated in tissue culture was sent to over 64 investigators. Over 5.5 million units of purified enzyme from the same virus was distributed to 273 researchers in approximately 500 shipments. As directed by OPR&L, the Cell Culture Laboratory distributed over 800 cell culture seed stocks to over 260 individual scientists in over 300 separate shipments, and analyzed over 150 individual cell cultures submitted by approximately 40 laboratories for cell-line purity and species identification. Furthermore, the Program repositories handled or shipped out over 45,000 individual items. In addition to these activities, the Office has

coordinated the distribution of a variety of resources to Russian, French, and Japanese scientists in keeping with formal U.S. or NCI international agreements with those countries covering the mutual exchange of cancer research materials. The materiel supplied included purified and concentrated viruses of murine, feline, avian, and primate origin; specialized viral proteins and antigens; normal and infected tissue culture cell lines; and a wide range of antisera prepared in a variety of host animals.

An additional responsibility of OPR&L is the monitoring and evaluation of the virus and reagent production and purification activities of Viral Oncology at the NCI-Frederick Cancer Research Center. OPR&L is also responsible for the management of the distribution of viruses, viral components, and cell cultures prepared at FCRC and provided to VOP collaborating laboratories. During this report period over 30,000 liters of virus materials were produced and purified, and over 25,000 ml. of various virus concentrates were released to investigators as directed by this Office. All Program requests for viruses available from FCRC have been met and approximately 2,000 ml. of virus concentrates remain available at the FCRC virus repository to meet additional needs.

The OPR&L also prepares an annual catalog listing and describing the research resources available to collaborating laboratories within the Program. These include viruses, antisera, human specimens, animal resources, special materials, and various services. Usually the information provided for each item includes designation, origin, and processing procedure. As in prior years, a revised new edition of the catalog was prepared this year and distributed to Program participants. Loose-leaf binding was retained to permit efficient updating of information, and the format and contents were again revised to expedite the recovery of items or services and reflect current research interests. This year's catalog again provided a detailed listing of the large numbers of animal tumor viruses available for distribution and of the antisera to special subvirion components. As noted in the catalog, an element of the OPR&L is concerned with the maintenance of a computerized central inventory for the sera, tumor tissue, cell out-growths, and other human specimen materials continuously being acquired by the Program. The central inventory facilitates matching investigators' requests for human materials with specimens available, regardless of the geographic location of the repository or laboratory at which it is stored.

Within the OPR&L, the Resources Data Management unit continued to assist in the automated retrieval and inventories of Viral Oncology Program resources, computer-systems planning, and automated analysis and management support. The automated inventories include the OPR&L serum collection, the human tissue collection, and the collections of the satellite resources systems. OPR&L maintains a computerized inventory of approximately 41,000 human sera specimens and approximately 7,000 freshly frozen malignant and normal human tissue specimens maintained in local and regional repositories. The RDM unit manages the continuous input, updating, and distribution data for these specimens stored for utilization by scientific investigators. Clinical and demographic data relevant to specimens distributed are provided to recipient investigators to assist in the analysis of test results. Support is also provided for previously developed and installed inventory systems in laboratories

participating as regional resource repositories. These include the Cell Culture Laboratory at the Naval Biosciences Laboratory, and the antisera production facility at the Huntingdon Research Center. Automated computer support is also provided for the specimen inventory of the Laboratory of Medical Oncology, NCI. Additionally, the RDM unit maintains an automated management system for data relevant to the distribution of research resources managed and administered by OPR&L. Also maintained is a data system for tracking the distribution of all virus products regardless of production source or recipient identity and location.

During this report period a comprehensive analysis of the overall resources production and distribution program was undertaken. To obtain complete annual estimates, calendar year 1977 was selected for review. The virus production and antisera preparation effort was shared by seven contracts whose funding represented 40% of the total resources budget. Of the specific agents produced during the year, 84% of the effort was for RNA viruses and 16% for DNA viruses. The inventory of available RNA tumor viruses included 56 agents of which 22 were in active production. The inventory of DNA viruses included 7 agents of which only 3 were in active production. The percent distribution of all resources was analyzed by the category of recipient. Approximately 35% of the viruses produced were distributed to NIH intramural investigators and 65% went to extramural recipients. Of the extramural recipients, 45% were VOP contractors and 20% were NIH grantees or had no specific affiliation. For antisera, approximately 69% went intramurally while 31% went extramurally. Fifty-eight percent of the extramural recipients were contractors and 16% were grantees. Only 18% of AMV reverse transcriptase enzyme was distributed to NIH laboratories while 81% went to non-NIH investigators throughout the world. Twenty-four percent were VOP contractors while 57% were grantees. Similar distribution patterns were found for virus-infected or non-infected cell cultures, avian materials, animals, human specimens, and other support efforts. A detailed analysis was also made of the support provided for RNA tumor virus studies, DNA tumor virus studies, co-carcinogenesis investigations, and similar categories. Fifty-five percent of the viruses distributed to VOP contractors was for RNA virus studies. Thirty-three percent of the virus preparation supplied to all other extramural laboratories for RNA virus work went to NCI grantees. Analysis of the distribution of materials for DNA virus studies by extramural laboratories showed greater support provided to NCI grantees than to VOP DNA studies contractors. This finding was valid not only for virus distributions, but for almost all resources and services provided for DNA virus research. Similar distribution and service patterns for support of the other virus research disciplines were developed. The comprehensive overview indicates that the resources program provides significant support not only to VOP intramural laboratories and VOP contractors, but also to NCI and NIH grantees and to the general scientific community.

Within the Office of Program Resources and Logistics, principal support is provided by two staff scientists, a systems analyst/computer programmer, and a secretary, who assist in responsibilities for the management of the collaborative contract program concerning research resources, and for the coordination of resources distribution.

Dr. Jack Gruber, Chief, OPR&L, VOP, DCCP
Dr. Garrett V. Keefer, Staff Scientist, OPR&L, VOP, DCCP
Dr. John S. Cole III, Staff Scientist, OPR&L, VOP, DCCP

AMERICAN TYPE CULTURE COLLECTION (NO1-CP-6-1047)

Title: Curatorial Preservation and Development of Reference-Grade Tumor Viruses

Contractor's Project Directors: Dr. Charles D. Aldrich

Project Officers (NCI): Dr. John S. Cole III
Dr. Garrett V. Keefer

Objectives: To biologically characterize and historically trace the origin of selected groups of tumor viruses, including avian, murine, feline, and primate, in order to develop and obtain reference-grade tumor virus materials. To serve as an archival repository for seed stocks of important virus materials from the Viral Oncology Program. To provide documented histories and characterizations of materials which have been provided in quantity to NCI collaborating investigators.

Major Findings: Receipt and characterization of oncogenic viruses from Program Resources and Logistics has continued. Data on RNA directed DNA polymerase of five murine and two primate viruses has been developed. Serological analysis of gs1 and gs3 antigen suggest a strong cross reactivity between squirrel monkey retrovirus and Mason-Pfizer Monkey Virus. The contractor reports a lack of correlation between *in vitro* assays of ecotropic viruses and leukemogenic activity *in vivo*, and an apparent transformation of euploid feline cells by Mouse Mammary Tumor Virus.

Significance to Biomedical Research and the Program of the Institute: Virus materials are supplied to investigators throughout the world by Program Resources and Logistics. It is important that highly characterized reference stocks of these viruses be available.

Proposed Course: This contract will continue for the duration of the approved project plan.

Date Contract Initiated: June 15, 1976

CHILD RESEARCH CENTER OF MICHIGAN (NO1-CP-3-3333)

Title: Inter- and Intraspecies Identification of Cancer Cells In Vitro

Contractor's Project Director: Dr. W. Peterson

Project Officer (NCI): Dr. John S. Cole III

Objectives: This contract provides Viral Oncology Program collaborating investigators with a service for rapid establishment or confirmation of species identity of cell culture systems.

Major Findings: During the past year, 358 cultures were received and evaluated by 706 different determinations of either immunofluorescent staining, glucose -6- phosphate dehydrogenase and lactic dehydrogenase isozymes and cytogenetic examinations, or combinations of these methods as necessary. Cell cultures of 23 different species, most of primate origin, were received from cancer research laboratories. Sixty-three (17.6%) of the cell cultures were found to be contaminated by cells of different species or different donor origin. Thirty-seven cell lines of human origin were incorrectly designated, including six with HeLa cell contamination. These figures have not changed materially since the last contract year; however, cell culture examinations increased by 55% during the same time period.

Significance to Biomedical Research and the Program of the Institute: In the search for oncogenic viruses, many cell cultures from the same or different species are used concurrently, which offer frequent opportunities for cross contamination. In multiple-species tumor transplantations, the species derivation of induced tumors sometimes comes into question. Generally, the significance of virus presence in tissue cells, the ability to grow virus, or the validity of virus isolator systems are all dependent upon the assurance of the identity of the cell cultures used.

Proposed Course: This effort will undergo a competitive selection during this year. The successful offeror will continue to provide service to Viral Oncology by rapidly determining interspecies and intraspecies identity of cell culture systems. Additionally, the contractor will extend and develop marker systems in accordance with needs for identification.

Date Contract Initiated: June 26, 1973

CHILDREN'S HOSPITAL MEDICAL CENTER (NO1-CP-6-1036)

Title: Supply of Pediatric Oncology Specimens

Contractor's Project Director: Dr. Gordon F. Vawter

Project Officer (NCI): Dr. Charles Boone

Objectives: To supply fresh and/or frozen pediatric tissue specimens for distribution to and study by collaborating cancer research laboratories.

Major Findings: The Children's Hospital Medical Center is a general pediatric hospital with active pediatric oncology units in surgery, radiation therapy, and medical oncology. The hospital's pathology department provides the diagnostic surgical and autopsy services to these units and to pediatric oncology patients of the Sidney Farber Cancer Center. Approximately 12 frozen specimens of tumor and related tissue from surgery and autopsy have been provided to the NCI repository for subsequent distribution to collaborating investigators.

Significance to Biomedical Research and the Program of the Institute: The supply of specimens from pediatric patients with malignant disorders is necessary to provide investigators in cancer research with material needed to carry out experiments to determine the possible viral etiology of cancer.

Proposed Course: This contract terminated March 9, 1978.

Date Contract Initiated: March 10, 1976

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-7-1000)

Title: Support Effort for the Coordinated Processing of Fresh and Viable Human Specimen Materials

Contractor's Project Director: Ms. Gretchen Stroemer

Project Officers (NCI): Ms. Wilma Varrato
Dr. Garrett V. Keefer

Objectives: The primary objective of this project is to assist the Office of Program Resources and Logistics (OPR&L), Viral Oncology Program, in the pick-up, handling, coordination, and delivery of human neoplastic and normal specimens for cancer research.

Major Findings: Biological materials handled by this project are available as by-product material from necessary clinical diagnostic and treatment procedures. These include tumor tissues, blood samples and other surgical

and autopsy tissues contributed from resource contractors in the United States and Canada. During the current period the contractor helped coordinate the distribution of more than 1,600 human specimens, supplied by NCI contract sources, thus permitting recipient research investigators to undertake intensive studies on the possible viral etiology of human cancer.

Significance to Biomedical Research and the Program of the Institute: It is not sufficient that sources of human tumor materials from cancer research exist; it is also necessary that the capabilities of these sources be coordinated with the needs of the investigators who require specific, and often fresh, specimens. This contractor, under the direction of the NCI project officer, serves as a direct interface between the user and supplier.

Proposed Course: This contractor will continue to assist OPR&L in the pick-up, handling, and delivery of human neoplastic and normal specimens.

Date Contract Initiated: October 8, 1976

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-7-1001)

Title: Computer Support Effort for OPR&L Resources Management

Contractor's Project Director: Mr. Mark Gladstone

Project Officers (NCI): Ms. Wilma Varrato
Ms. Kathryn Hancock

Objectives: To assist in processing, storage, and retrieval of data associated with research resource materials of the Viral Oncology Program.

Major Findings: During the last year, the contractor continued to provide computer support to the Office of Program Resources and Logistics. Efforts were directed toward: a) the design and development of new and revised systems for the management of the collection, storage, and distribution of research systems; b) data entry to support the operation of existing systems; and, c) the production of reports from the data bases for the various systems.

Significance to Biomedical Research and the Program of the Institute: Computerization of resources data makes it possible for the NCI Office of Program Resources and Logistics to exercise close control over the inventory of viruses, sera, human tissues, and other materials provided by the VOP and used in cancer research. In addition, computerization makes it possible to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction, thereby, permitting rapid response to the needs of the Viral Oncology Program while avoiding resource excesses or shortages.

Proposed Course: The contractor will provide systems analysis, computer programming, and management of data as required by OPR&L. The contractor will extend and refine the central inventory system as necessary to meet the ever-changing requirements for materials used in cancer research. Appropriate management reports will be generated as required by the Viral Oncology Program.

Date Contract Initiated: March 1, 1977

ELECTRO-NUCLEONICS LABORATORIES, INC., (NO1-CP-2-3249)

Title: Large-Scale Production of Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. John S. Cole III

Objectives: To provide a service related to the isolation, large-scale production, concentration, and assay of oncogenic viruses of animals and potentially oncogenic viruses of humans. Production and quality control involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: During this report period the contractor processed 8,044 liters of virus-containing fluids harvested from several tissue culture systems and, as directed by the Project Officer, distributed the purified virus concentrates and cells to the Program Resources and Logistics repository and to individual investigators involved in a variety of research projects. The flexibility of the program was emphasized in several ways. The production of AKR virus was continued and a procedure for obtaining AKR virus with a high yield of gp70 was introduced. This new procedure was included on an alternate basis with the regular production of AKR in order to satisfy a number of requests for virus with high yields of gp70. The production of Moloney leukemia virus and Gross leukemia virus was terminated and the production of four xenotropic viruses - Balb virus 1, Balb virus 2, NZB:MuLV and NIH:MuLV - was increased.

The xenotropic viruses now comprise about half of the contractors total required production volume. The cell lines yielding xenotropic virus only produce about one-tenth as much virus, compared to the exogenous viruses. Therefore, considerably increased virus-tissue culture fluid volumes are required to maintain equivalent quantities of concentrated xenotropic virus for the various recipient investigators.

The quality of the contractor's virus products is frequently monitored by gel electrophoretic analysis of viral RNA (the spectrophotometer scanning of

the gel detects approximately one microgram of RNA) and by PAGE analysis for the viral structural protein profile and envelope glycoprotein content.

Significance to Biomedical Research and the Program of the Institute: In order to carry out important research on the biochemistry and biophysics of oncogenic animal viruses, it is imperative that large quantities of concentrated virus be available for analysis. This contract helps meet this need with oncogenic animal viruses that have been produced under rigidly controlled conditions, and also serves to find the best means of producing and concentrating large quantities of new candidate human cancer viruses as they are discovered.

Proposed Course: The contractor will continue to provide regular AKR virus, AKR virus with high yields of gp70, and four xenotropic murine viruses; and will provide other special preparations as requested by the NCI project officer. The contractor will continue to develop and optimize methods for producing the highest virus yield with consistantly high biological qualities, with the flexibility to quickly accommodate shifts in Program requirements.

Date Contract Initiated: March 27, 1972

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-4-3334)

Title: Virus Processing and Production Facility

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officers (NCI): Dr. George Vande Woude
Dr. Jack Gruber

Objectives: The purpose of this contract is to establish and maintain a facility capable of a variety of batch-scale production of animal oncogenic and/or suspected oncogenic viurses; and also for the processing of large volumes of virus containing fluids supplied by other NCI-designated laboratories or investigators.

Major Findings: During this report period the contractor processed and characterized a total of 9,119 liters of virus-containing fluid. Of the total virus fluid processed and characterized, they have propagated 4,711 liters of virus-containing cell culture fluids. Thirty-three different type C oncornaviruses and one type D oncornavirus were propagated, processed and characterized during this period as directed by the Project Officer. The purified virus concentrate and packed tissue cells were distributed to the Project Officer and to individual investigators involved in a variety of research projects. The contractor also has produced and processed 998 equivalent liters of several herpesviruses in Vero cells, and Herpesvirus saimiri in OMK cells utilizing polyethylene glycol precipitation and

Renografin gradient centrifugation. In addition the contractor develc a Freon-113 extraction and centrifugation procedure to recover nucleo from the herpesvirus infected cell packs.

Significance to Biomedical Research and the Program of the Institute: This project provides cooperating investigators with the opportunity to process large quantities of virus-containing tissue culture fluids, as well as smaller quantities of a variety of viral agents of special interest. There is a strong need for this effort within the Program since it provides a special flexibility for intramural investigators.

Proposed Course: The work described above will be continued at approximately the same level of effort, and be flexible enough to support the changing needs of the Viral Oncology Program and its collaborating investigators.

Date Contract Initiated: June 15, 1974

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NO1-CP-3-3343)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. Lea I. Sekely

Objectives: To monitor the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to VOP collaborators for transplantation, tissue culture and virus isolation studies.

Major Findings: During this report period, a group of 56 aging and/or irradiated rhesus monkeys and progeny were monitored by daily observations and tri-annual physical and hematologic evaluation. These examinations specifically concentrated on the developing neoplasms. During the most recent survey, 17 animals were classified as slightly to moderately emaciated and skin and/or subcutaneous nodules or masses were present in 15 animals. One animal had a large mass in the thigh that has been characterized as a lipoma; two animals have basal cell carcinoma of the skin; two animals continue to have a very large mass in the lower abdomen in the region of the uterus; and one animal continues to have a palpable mass in the region of the liver.

Three of the four adult animals that died during the report period had tumors and one animal had two separate primary tumors. Neoplasms encountered included an intestinal adenocarcinoma, a transitional cell carcinoma of the kidney pelvis, a sarcoma of the hip region, and thyroid adenomas.

During this report period, 149 specimens were shipped to Viral Oncology Program investigators and collaborators. These specimens included 119 serum samples, six whole blood samples, and 24 tissue specimens (includes samples from one sarcoma). Preliminary data reported by these collaborators indicate that at least nine of these animals have demonstrable titers to Mason-Pfizer Monkey Virus.

Observations in this group of animals continue to confirm their value as a source of nonhuman primate tumor materials, as 17 of 36 animals (47.2%) that have died in this group have had neoplasms.

Significance to Biomedical Research and the Program of the Institute: The VOP conducts collaborative projects for the study of relationships between the etiologies of tumors of various primates. This project provides tumor tissues and other important specimens from aging, irradiated subhuman primates for research within the VOP. Malignant changes in these irradiated primates may provide useful information which might be applied to humans, who are also subjected to similar physical stresses.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations. All tumors which develop will be evaluated by the contractor using light and electron-microscopy. Specimens of these tumors will be made available to VOP investigators. In addition, a program is underway to evaluate the incidence of leukemia or other tumors in infants with aging and irradiated parents.

Date Contract Initiated: May 1, 1971

EMORY UNIVERSITY, SCHOOL OF MEDICINE (NO1-CP-6-1051)

Title: Supply of Human Tumor Specimens and Related Material for Cancer Research

Contractor's Project Director: Dr. Douglas R. Murray

Project Officers (NCI): Ms. Wilma L. Varrato
Dr. John S. Cole III

Objectives: To supply by-product surgical human mammary tissue and serum to NCI for cancer research utilization.

Major Findings: During the past year, the contractor supplied 56 frozen tissue specimens, 22 fresh viable tissue specimens, and 104 sera specimens. The major portion of the specimens were malignant breast tumors. Other diagnoses included osteogenic sarcoma of lung, adenocarcinoma of rectum, sarcoma of lung, and benign tumors.

Significance to Biomedical Research and the Program of the Institute: Finding of virus-like particles as well as 70S RNA and reverse transcriptase in breast cancer tissue by several investigators within VOP strongly suggest that a virus may be associated with human breast cancer, therefore, breast neoplastic specimens are of critical importance in studies of the possible viral etiology of human mammary cancer.

Proposed Course: To carry out the activities described above.

Date Contract Initiated: June 28, 1976

ENVIRO CONTROL, INC. (NO1-CP-6-1021)

Title: Monitoring of Biohazard Containment Facilities

Contractor's Project Director: Dr. Robert W. McKinney

Project Officer (NCI): Dr. Alfred Hellman

Objective: In collaboration with the Viral Oncology Office of Biohazard Safety (OBS), NCI, the contractor: performs safety inspections of virus production facilities which provide resources to NCI to determine compliance with relevant safety standards; performs biological safety and environmental control surveys to contract research facilities for the purpose of providing guidance in biohazard control technology with aim of reducing accident potential and promoting the integrity of experimental systems by protecting them from contamination; evaluates the effectiveness and accomplishments of previous OBS safety program and recommends means to improve the OBS control program; provides safety, environmental microbiology, and engineering consultation to NCI laboratories and to other parties seeking assistance from OBS; and procures and installs essential safety equipment needed by Program scientists involved in cancer research. Through requests from the Office of Research Safety, OD, NCI, the contractor also assists other parties by providing technical safety information and guidance.

Major Findings: During the period covered by this report, 20 site visits were were conducted, which involved 26 separate contracts. Five of the contracts were resources efforts and 19 were research. In addition, visits were made to provide consultation regarding renovations to a facility as well as a consultation to a Food and Drug Administration laboratory.

Support was provided to the Office of Research Safety, NCI and the Research Facilities Branch, NCI in the review of plans and specifications for ten proposed or active laboratory construction projects. In addition, two courtesy consultations were provided. These reviews and consultations were conducted for the purpose of evaluating the adequacy of design and specifications in relation to biohazard containment and environmental control.

Work was continued on the development of modifications for two items of safety equipment. These were: (1) a primate containment system; and, (2) a small animal necropsy cabinet. The preparation of review documents was continued and during this report, reviews were initiated in the subject areas of: (1) Face Velocity Criteria for Chemical Type Fume Hoods; (2) Constant Air Volume Control Systems; (3) Incineration; and, (4) Storage of Chemicals.

Support to the NIH Recombinant DNA Research Program was continued. This has been expanded to include the development of Class III cabinet systems utilizing surplus units from the Ft. Detrick laboratories. These cabinet systems will be installed in laboratories of the NCI.

Significance to Biomedical Research and the Program of the Institute: This contract contributes biological safety and environmental control expertise to the VOP and to the general scientific community. This expertise is used to improve the quality and safety of the cancer research laboratory environment. The contractor functions as an important support to the NCI Office of Biohazard Safety.

Proposed Course: The contractor will continue to provide technical assistance to the VOP contractors on problems of environmental control, personnel safety, and product protection. All recommendations will be based on compliance with accepted engineering design, biological safety and environmental control practices.

Date Contract Initiated: September 29, 1976

FLOW LABORATORIES, INC. (NO1-CP-5-3566)

Title: Animal Holding Facility to Support Intramural Research on RNA Tumor Viruses

Contractor's Project Director: Mr. Paul J. Cote, Jr.

Project Officers (NCI): Dr. John W. Pearson
Dr. Garrett V. Keefer

Objectives: To support NCI intramural research activities being conducted in the Viral Immunotherapy Section, Laboratory of RNA Tumor Viruses. The nature of the intramural activities requires the long-term holding of aged mice, rats, and guinea pigs for the study of the effects of drug therapy, surgery, vaccines, non-specific immune stimulators and interferon used alone or in combination against viral-associated transplantable tumor lines.

Major Findings: During this report period, the contractor received and held a total of 3,699 animals, principally guinea pigs and rats. These animals were received as weanlings, aged for one month, and monitored for an additional

two to seven months to support the following areas of experimentation: 1) the application of drugs or surgery alone or in combination with immunotherapy in aged guinea pigs or rats exhibiting neoplasia; 2) the long term immunization of guinea pigs to determine the antigenicity of tumors and leukemia either occurring spontaneously or induced by chemicals or viruses; 3) in vivo efforts to monitor cellular suppression following drug therapy or surgery; and 4) long term observation for the development of metastatic lesions following surgical removal of the primary tumor. In addition, weekly shipments of leukemic and normal bloods, serum, plasma, peritoneal exudates, lymphatic tissue, tumor cells, and other organs have been sent to various NCI investigators.

Significance to Biomedical Research and the Program of the Institute: Existing facilities and space for new facilities devoted to the holding of small laboratory animals at the NIH reservation are extremely limited, with the result that necessary in vivo experimentation are severely curtailed. This contract provides holding facilities for small laboratory animals with a back-up laboratory support service that enables intramural investigators to continue research of high priority to the goal of the VOP.

Proposed Course: It was decided to fund this effort only through September 30, 1978 due to uncertainty as to the ability of the contractor to provide the service at his new facility. Contract expires September 30, 1978.

Date Contract Initiated: June 15, 1971

FLOW LABORATORIES, INC. (NOI-CP-6-1000)

Title: Repository for Storage and Distribution of Reagents, Sera, and Tissue Specimens

Contractor's Project Director: Mr. Rodney Miller

Project Officers (NCI): Dr. Jack Gruber
Dr. Garrett V. Keefer

Objectives: This contract provides a low temperature storage facility for specially developed biological reagents and clinical specimens. The facility receives, inventories, and stores for Program use, research reagents, and normal and neoplastic human specimens, for further distribution to designated scientific investigators as authorized by OPR&L, NCI. Accurate inventories and records of distribution are provided to NCI. Additionally, the contractor applies adequate safety measures to maintain the integrity of all materials stored and shipped.

Major Findings: During the current period, the contractor made 462 shipments of viruses, viral reagents, and sera which comprised a total of 16,581 vials

of material. The facility received 360 shipments of similar material which comprised 28,385 vials. All incoming shipments were carefully checked for damage in transit and were cataloged before being placed in the low temperature repository. During the same period, 3,155 tissue specimens were received. All tissue specimens were examined by a pathologist, classified as to tumor and tissue type, and cataloged and stored for further distribution.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible, adequately characterized resource materials. The laboratory, storage, and shipping facilities operated under this contract enable collaborating investigators to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: It is anticipated that the activities of this contract will continue to provide rapid and flexible support to changing needs of the Viral Oncology Program and its collaborating investigators.

Date Contract Initiated: June 22, 1965

HEALTH RESEARCH, INC. (RPMI) (NO1-CP-4-3392)

Title: Supply of Blood and Tissue Specimens from Patients with Malignancies

Contractor's Project Directors: Dr. John W. Pickren
Dr. Joseph Sokal

Project Officer (NCI): Ms. Wilma Varrato

Objectives: To collect tissues and blood samples from adults with malignancies for use by cancer researchers within the Viral Oncology Program.

Major Findings: Health Research, Inc. is located at the Roswell Park Memorial Institute in Buffalo, New York, one of the major centers for treatment of cancer in this country. During the past year 234 specimens from autopsy were shipped to the NCI respository for distribution to cancer research investigators. Tumors were submitted from 79 different diagnoses. The most common tumors were: carcinoma of lung; carcinoma of breast; and leukemia. Most of the tumors represented metastases in the liver, lung, lymph nodes and bone. Moreover, 107 surgical specimens were shipped to the NCI resources processing laboratory for rapid distribution. The surgical and blood specimens shipped were materials remaining from standard surgical and clinical procedures after the necessary portions for diagnostic purposes were obtained.

Significance to Biomedical Research and the Program of the Institute: A major goal of the VOP is to isolate, propagate, characterize, and identify candidate human cancer viruses. Of paramount importance in the efforts to

reach this goal is the continued availability of clinical specimens from patients with cancer. This institution has available a large number of cancer patients and, on a continuing basis, is in an excellent position to help meet program needs for human neoplastic specimens.

Proposed Course: It is anticipated that specimen acquisitions will be continued to meet program needs.

Date Contract Initiated: June 22, 1972

HEKTOEN INSTITUTE FOR MEDICAL RESEARCH (NO1-CP-4-3344)

Title: Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases

Contractor's Project Director: Dr. Paul Szanto

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: The objective of this contract is to provide sterile, viable, and freshly frozen normal or neoplastic human specimens to NCI for distribution to collaborating laboratories for biological, chemical, and virological cancer research investigations.

Major Findings: The Division of Pathology of the Hektoen Institute for Medical Research is affiliated with the Cook County Hospital in Chicago, Illinois. Last year this hospital admitted approximately 50,000 patients and the Pathology Division received over 20,000 specimens for diagnosis; in addition, there were autopsies performed. The principal investigator, the Director of the Pathology Division, made available a large number of human specimens not needed for clinical or pathologic diagnosis to investigators collaborating with NCI. A total of 702 sterile tissue specimens were shipped to the NCI resources processing center. Diagnoses represented carcinoma of the breast, ovary, stomach, kidney, esophagus; malignant lymphoma and Hodgkin's disease; liposarcoma, and a number of benign neoplasms and normal tissues. A total of 606 tumor and organ specimens were also shipped to the NCI repository. The tumors represented included carcinoma of the bladder, carcinoma of the cervix, lymphomas, leiomyosarcoma, angioma, and carcinoma of the pancreas. Normal adult and fetal tissues, and benign tumors were also supplied.

Significance to Biomedical Research and the Program of the Institute: A continuous supply of appropriate human tissues from patients with cancer is vital to cooperative cancer research investigations. In order to carry out important studies on the biochemistry and immunology of suspected oncogenic human viruses, it is imperative that large quantities of human malignant tissues be available for analysis.

Proposed Course: This contractor will continue to supply tissue specimens from patients with neoplastic disease and from normal individuals.

Date Contract Initiated: June 17, 1974

HOSPITAL FOR SICK CHILDREN (NO1-CP-6-1040)

Title: Normal and Leukemic Human Tissue Collection

Contractor's Project Director: Dr. Peter D. McClure

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: To gather serum, whole blood, and tumor specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls, during the course of normal clinical procedures.

Major Findings: During the year the contractor collected serum samples from new patients with leukemia and from patients on long-term follow-up for use by Viral Oncology Program collaborating investigators. Twenty-seven tissues, 28 buffy coats, and 168 serum samples were shipped to the NCI repository. In addition, 52 surgical specimens and 27 fresh whole blood specimens were sent to the NCI resources processing laboratory for distribution.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor supplies many vital serum specimens to the VOP not readily available elsewhere. The contractor also provides an important service to Program by providing numerous samples of fresh and frozen buffy coats and tissue specimens.

Proposed Course: The contractor will continue to collect pediatric solid tumor tissue, sera, and leukemic white cells.

Date Contract Initiated: June 1, 1976

HUNTINGDON RESEARCH CENTER (NO1-CP-3-3223)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger Wilsnack

Project Officers (NCI): Dr. John S. Cole III
Dr. Garrett V. Keefer

Objectives: The objectives of this contract are to develop, produce and characterize antisera to oncogenic viruses and their components for use in the Viral Oncology Program. In addition, antisera to globulins of various animal species and the T-antigen of SV40 are produced.

Major Findings: During this reporting period the contractor produced and characterized antisera against a wide array of antigen encountered in cancer research, and added several new antisera to oncornavirus proteins to his inventory. Among these new antisera were anti-MMTV gp52, anti-MMTV p12, anti-Balb Virus 2 p12, anti-RLV p15(e) and anti-Baboon endogenous virus reverse transcriptase. During this reporting period, the contractor distributed over 30,500 ml. of antisera to over 300 collaborating investigators in the VOP.

Significance to Biomedical Research and the Program of the Institute: The antisera developed and produced by this contract are important tools in cancer research. The close collaboration of the project with viral oncology research programs results in significant usefulness not only to program but to the entire research community.

Proposed Course: This effort will be subject to competitive selection in the current year. The successful offeror will continue to provide for the needs of cancer oriented investigators.

Date Contract Initiated: June 2, 1963

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CP-7-1032)

Title: Supply of Japanese and Asiatic Rodent and Primate Materials

Contractor's Project Director: Dr. Yoji Ikawa

Project Officer (NCI): Dr. George Todaro

Objectives: To provide specialized Asiatic animal materials for cancer research studies on the isolation and characterization of endogenous viruses.

Major Findings: During the eight months after the initiation of this new contract, 26 colonies of newly trapped Japanese and Asiatic rodents were established at the animal laboratory of the National Institute of Genetics, Mishima, and that of the Cancer Institute, Tokyo. Cell culture lines have been established from the viscera of the newborn rodents of seven different subfamilies, and those culture lines have been frozen and stored for future distribution. Reverse transcriptase activity has been examined in the cultures described above with and without viral induction by BUdR, etc., with negative results. Complementary DNAs for mouse and rat endogenous viruses have been prepared for further genetics of endogenous viruses among Japanese wild

rodents. Accidental HVJ infection to some colonies at the N.I.G. animal laboratory has been recovered by introduction of new colonies and separation of the colonies. A group of collaborating scientists will depart soon for the trapping of wild rodents for future activities.

Significance to Biomedical Research and the Program of the Institute: Asian rodent species release a number of endogenous type-C viruses which seem related to horizontally transmitted viruses isolated from some nonhuman primates. These agents are similar to animal oncogenic viruses. There is a high probability that other similar viruses can be isolated from Asiatic animal resources to clarify the significance of agents already characterized or which will be significant in themselves. A resource of this nature can help establish the original source of some oncogenic primate viruses.

Proposed Course: This acquisition effort will continue for the duration of the approved project plan.

Date Contract Initiated: August 1, 1977

JEWISH HOSPITAL AND MEDICAL CENTER (NO1-CP-4-3251)

Title: Supply of Human Specimen Material from Patients with Malignancies

Contractor's Project Director: Dr. Harvey Dosik

Project Officer (NCI): Dr. Jack Gruber

Objectives: To provide tissues and serum specimens from patients with various types of malignancies and genetic disorders related to cancer, as well as from patients with disorders of a non-malignant nature, to collaborating investigators for cancer research.

Major Findings: The Hematology Service of the Jewish Hospital is the largest in Brooklyn with approximately 1,100 new in- and outpatient referrals per year. This large population of patients, combined with the collaborative efforts of five other Brooklyn hospitals, permitted the contractor during this period to provide more than 1,500 serum samples to the Program. In addition, more than 1,000 sterile and non-sterile solid tumor specimens were collected from biopsy and autopsy procedures for shipment to the NCI resources processing center and the NCI repository. In addition 141 whole blood, buffy coat and other body fluid specimens were made available to NCI collaborating investigators.

Significance to Biomedical Research and the Program of the Institute: Fresh and frozen tissue and sera from patients with malignant disorders, related disorders, and individuals without malignancies are necessary for experiments to determine the etiology or etiologies of various malignancies.

Proposed Course: It is anticipated that the contractor will continue to supply normal and neoplastic tissues from chromosome abnormalities and various malignancies as determined by the needs of the Program.

Date Contract Initiated: March 1, 1973

JOHNS HOPKINS UNIVERSITY (NOI-CP-3-3245)

Title: Pediatric Tumor Resource

Contractor's Project Director: Dr. Herbert Kaizer

Project Officers (NCI): Dr. Charles Boone

Objectives: The goal of this contract is the collection and distribution of biological materials related to pediatric malignant diseases.

Major Findings: This contract effort gathers clinical by-product material from an active pediatric oncology program at the Johns Hopkins Hospital. Its geographic proximity to the National Cancer Institute makes the effort an ideal resource of tumor material for a variety of studies being undertaken by the NCI Viral Oncology Program.

For the period of this report a total of 121 specimens, including 43 tissue specimens, from 30 different patients were transmitted. In general those materials included sera from patients and family members, body fluids containing tumor cells and solid tissues that became available from biopsy or necropsy, including tumor and uninvolved organs or adjacent tissues. Most solid specimens have been in excess of one gram and all materials, except those from necropsy, were transmitted sterilely within a few hours. Of special interest were two placentas from mothers who had previous children with cancer and biopsy and necropsy material from a child with four independent and different neoplasms. The contractor also started a collection of sera obtained from oncology clinic patients and their families during the course of clinical activities. These sera have been transferred periodically in large batches to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: Detection, treatment, and prevention of human cancer requires an adequate determination of its etiology. Viruses have been implicated as possible causative agents in human cancer. Unfortunately, a lack of sufficient tumor material from patients in the pediatric age group limits investigations for oncogenic viruses that may have been vertically transmitted from mother to child. Materials from this contract will help make possible these imperative human studies.

Proposed Course: It is anticipated that this contractor will continue to supply unique pediatric specimens to NCI.

Date Contract Initiated: March 1, 1973

LIFE SCIENCES, INC. (NO1-CP-3-3291)

Title: Production of Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officer (NCI): Dr. John S. Cole III

Objectives: The objectives of this project are the large-scale in vivo production of BAI strain A avian myeloblastosis virus (AMV) and the preparation and distribution of significant quantities of AMV reverse transcriptase enzyme.

Major Findings: During the current period, the contractor obtained a total of 718 grams of AMV from the plasma of 360,000 chicks. Of this total amount of virus, 63 grams were shipped as frozen plasma, 203 grams were shipped as fresh plasma or virus pelleted by centrifugation, 58.1 grams were used as an inoculum to produce more AMV, and 453 grams were used to prepare reverse transcriptase enzyme (RDDP).

Sixty-four investigators were recipients of plasma containing a total of 265 grams of AMV during this period; nine investigators received approximately 2,225 ml. of myeloblast cells of myeloblastic chicks; and over five million units of RDDP were sent to 273 scientists. Recipients of these materials were in laboratories throughout the world.

Significance to Biomedical Research and the Program of the Institute: One of the major objectives of the VOP is to explore fully all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced in vivo under controlled conditions which permit the study of the immunology, virology, biochemistry, and therapy of the tumor virus complex. Moreover, BAI Strain A avian tumor virus is the only RNA C-type virus which is at present available in large enough quantities to permit exhaustive investigation into the biochemical makeup and behavior of both the virus and its components. As such, it represents an important model for the C-type viruses of higher animals and is an essential tool in the search for cancer viruses in man. Future studies will depend upon large quantities of concentrated virus, which the contractor is uniquely in a position to supply.

Proposed Course: It is anticipated that the contractor will continue to meet requests for avian myeloblastosis virus and will continue the preparation of significant quantities of AMV reverse transcriptase enzyme.

Date Contract Initiated: April 19, 1971

LIFE SCIENCES, INC. (NOl-CP-6-1005)

Title: Production and Maintenance of Selected Reagent Grade Specific Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. David McB. Howell

Objectives: To produce specific-pathogen-free (SPF) animals for cancer research. SPF animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts.

Major Findings: During the report period one outbred expansion colony of isolator-derived Balb/c mice contained approximately 750 female breeders that supplied 633 timed-pregnant and 3,221 young adults to user laboratories. The outbred Balb/c colony was begun with a small, inbred Balb/c stock of ten pairs maintained in a Reyniers isolator. A Caesarean-derived nucleus of Balb/c nude, athymic mice was expanded to 50 male homozygous nudes and 185 female heterozygous breeders, that supplied 1,036 nude mice to various investigators. One cubicle reared expansion colony of 575 female, Caesarean-derived, outbred NIH Swiss mice supplied 565 timed-pregnant and 720 young adult animals to user laboratories. An NIH Swiss nude mouse colony of 1,625 heterozygous female breeders, divided into five expansion colonies, supplied 8,235 homozygous weanlings, 3,194 heterozygous littermates and 208 time-bred females. Antibody testing of sera from Balb/c, NIH Swiss, and heterozygous females in the nude breeder colonies indicated that all lines are free of six common murine viruses.

A total of 12,500 fertile quail eggs were supplied to user laboratories from a highly selected Japanese quail flock of 355 breeders maintained in a primary barrier cubicle. The isolator-derived flock was developed through elaborate trap nesting methods and recent monitoring in this laboratory indicates the line is free of common avian pathogens, including avian leukosis viruses and Marek's herpesvirus. An isolator-derived, pedigreed chicken flock of 475 breeders produced a total of 7,459 fertile eggs (7,038 C/E, 291 C/O, 130 C/B), 1,644 embryonated eggs and 1,751 chicks (1-3 days of age). The pedigreed flock was monitored at regular intervals during the laying cycle and following retirement at 11-12 months of age. Extensive monitoring indicates

the flock contains no avian leukosis virus, Marek's herpesvirus or other common avian pathogens. Regular phenotyping of eggs from pedigreed stock indicates the presence of C/E, C/O and C/B types. In subsequent generations, the phenotype may change from one type to another thus requiring continual monitoring ~~in each new flock to clarify status of each~~ pedigreed bird. Efforts are continuing to increase the number of chickens in the flock with C/O pedigree.

Significance to Biomedical Research and the Program of the Institute: This contract provides VOP investigators with genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with a minimal danger of interference from other contaminating, adventitious micro-organisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: This service-type contract for the production of reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of VOP activities as they occur.

Date Contract Initiated: February 8, 1968

LITTON BIONETICS, INC. (NO1-CP-6-1006)

Title: Operation of a Facility to Provide and Maintain Subhuman Primates for Cancer Research

Contractor's Project Director: Dr. John Cicmanec

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. Jack Gruber

Objectives: The objective of this contract is the maintenance of monkey breeding colonies and laboratories necessary for inoculation, care, and monitoring of primates.

Major Findings: During this report period efforts continued to provide both New World and Old World primates and biological materials from these animals for use by cancer research investigators. The New World breeding colony now has 458 animals representing twelve species; 131 live births occurred in the breeding colony during this report period including 22 infants born in the cotton-topped marmoset (Saguinus oedipus) colony. The cotton-topped marmoset has been identified as an endangered species, which means they can no longer be imported into this country.

Approximately 208 macaques comprising three species were in the Old World breeding colony. A small breeding colony of gibbon apes (Hylobates lar)

was also maintained. One hundred and eight live births occurred in the Old World breeding colony, which included five births in the gibbon ape breeding colony. One hundred and seventy-seven animals were assigned to 22 active special studies during this report period.

In support of these efforts, personnel in the Department of Pathology performed 200 complete necropsy procedures involving the preparation of 2,535 microslides (42 with special stains). The surgery team performed 24 major procedures to support the program, and the Department of Microbiology continued its support by performing specimen cultures, antibiotic sensitivity testing, and environmental monitoring. The Hematology Section performed 1,495 complete blood counts. Thirty-nine coagulograms, 1,432 clinical chemistry procedures, and 13 bone marrow biopsies were processed. Routine monitoring and cataloging of specimens continued in the Parasitology Section. A total of 296 tissue or serum samples were transferred or sent to outside investigators.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human cancer viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes.

Proposed Course: This contract will continue the following activities: the maintenance of breeding colonies of nine different species of New World primates; production of 100 to 150 infants of various species of both Old World and New World primates for use in experimental studies; continuing efforts in the establishment of a breeding colony of gibbon apes; and the long-term holding and study of experimental animals inoculated by collaborating investigators. All of the systems needed for the production, hand rearing, isolation, and proper care of the primate species represented are included within this program.

Date Contract Initiated: February 12, 1962

LITTON BIONETICS, INC. (NO1-CP-6-1039)

Title: Holding Facility for Small Laboratory Animals

Contractor's Project Director: Dr. Peter Golway

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The objective of this contract is to provide space for the holding and experimental manipulation of small laboratory animals for the direct support of Viral Oncology Program intramural investigators.

Major Findings: During this report period the contractor has received, maintained, monitored and experimentally manipulated a total of 66,660 mice, 836 rats and 84 rabbits in support of three intramural investigators. An average of 3,258 mice were received each month. Experimental procedures that have been performed included tumor protection tests, foot-pad assays, potency and safety testing of human skin test antigens, application of methylcholanthrene to induce squamous cell carcinoma, and the passage of seven virally induced transplantable tumors. The breeding colony of C3H/f/B and C3HA^{vy}/B strains of mice has increased in size from 72 to 488 animals. Strain C3HA^{vy}/B mice have a high incidence (90%) of mammary tumors that usually appear in the females at 15 months of age. Females are retired after parturition and held until tumors appear.

Significance to Biomedical Research and the Program of the Institute: Existing facilities and space for new facilities devoted to the holding of small laboratory animals at the NIH reservation are extremely limited, with the result that necessary *in vivo* experimentation had been severely curtailed. This contract provides holding facilities for small laboratory animals with back-up laboratory support services that enable intramural investigators to continue research of high priority to the goals of the VOP.

Proposed Course: This support type contract that provides holding space for small laboratory animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet the changing needs of intramural investigators.

Date Contract Initiated: June 25, 1976

MAYO FOUNDATION (NO1-CP-7-1031)

Title: Human Specimen Acquisition for Cancer Research

Contractor's Project Director: H. C. Hoagland, M.D.

Project Officers (NCI): Dr. John S. Cole III
Dr. Jack Gruber

Objectives: To acquire byproduct human material consisting mainly of serum samples, and of white blood cell packs from patients undergoing leukophoresis as a part of the treatment regimen for blast crisis in leukemia.

Major Findings: Several hundred serum specimens and 12 leukopacks have been provided during the first six months of the contract effort. These have been utilized in a major ongoing study of the human cancer problem. It is anticipated that the contractor will fully meet his commitments by the anniversary date of the contract.

Significance to Biomedical Research and the Program of the Institute: Rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at the Mayo Clinic in Rochester, Minnesota provides cooperating investigators with sufficient numbers of specimens from leukemic patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: It is anticipated that this contract will continue for the duration of the approved project plan.

Date Contract Initiated: September 27, 1977

MELOY LABORATORIES, INC. (NO1-CP-4-3263)

Title: Mouse Mammary Tumor Virus Production Facility

Contractor's Project Director: Dr. Bhalchandra Diwan

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To propagate, concentrate, and distribute murine mammary tumor virus (MTV) to collaborating VOP investigators; to perform immunological and biological assays for the detection and quantitation of MTV; to produce MTV from tissue culture; and to develop improved methods for the propagation and detection of MTV and MTV antigens.

Major Findings: The primary purpose of this contract is the production of quality reagents for the study of the mouse mammary tumor virus system as a model for the further examination of the human breast cancer problem. The current RIII mouse colony presently consists of approximately 1,600 breeding females, of which 1,282 are in the dairy facility. This number of animals is sufficient to produce approximately 200 ml. of milk per week.

A total of 17.2 liters of RIII milk was collected during the report period and 128 virus purification runs were completed yielding a total of 165 ml. of purified mammary tumor virus. A total of 113 mammary tumors have been recorded and distributed. In addition the following antisera have also been distributed: rabbit anti-C3H-MMTV, goat anti-MMTV gp52 (RIII), and goat anti-MMTV gp27 (RIII).

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. A major effort of the VOP is directed toward determining the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining information on the *in vivo* isolation and propagation of a murine mammary tumor virus (MMTV) and was an early source of large quantities of

purified MMTV. However, tissue culture-derived C3H MMTV has been available to collaborating investigators in sufficient quantities to meet all anticipated needs. The MMTV propagated in cell culture appears to be a cleaner preparation and is seemingly free of endogenous type C virus contaminants.

Proposed Course: This contract terminated April 12, 1978.

Date Contract Initiated: January 13, 1974

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-6-1038)

Title: Acquisition of Human Specimens for Use in Cancer Research

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officers (NCI): Dr. Jack Gruber
Dr. Garrett V. Keefer

Objectives: To collect sera and tissues from human subjects with neoplastic tumors to be used in cancer research studies by collaborating investigators within the Viral Oncology Program.

Major Findings: This contract represents one of the major acquisition and distribution efforts for the various human neoplasm specimen material so necessary to the multifaceted research programs currently in progress that are seeking to ascertain the possible relationship of viruses to the etiology of human cancer. During the report period, the contractor provided to collaborating laboratories over 2,500 specimens, including 1,339 tissues, 1,086 bone marrow samples, and 301 whole blood and white cell packs. In addition, 1,082 serum specimens were shipped to the NCI repository for further distribution.

Significance to Biomedical Research and the Program of the Institute: Rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City provides cooperating investigators with sufficient numbers of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: It is anticipated that this contractor will continue to supply material according to the needs of the Program.

Date Contract Initiated: March 1, 1971

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-3-3288)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Operation of a Service Laboratory

Contractor's Project Director: Dr. Michael Collins

Project Officers: Dr. Garrett V. Keefer (NCI)
Dr. Wallace P. Rowe (NIAID)

Objectives: To develop reagents and tests for the detection of rodent viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations; and to assist in the characterization of the gene-dependent expression of murine leukemia.

Major Findings: The contractor operates a murine virus serodiagnostic and viral diagnostic laboratory for NCI. During this report period the serodiagnostic laboratory received 6,680 sera from mice, rats, hamsters and guinea pigs on which 27,820 serological tests were performed. A total of 107 animal tissues, transplantable tumors, ascites, cell cultures and viral reagents were tested for murine viral contamination by the MAP or RAP procedure and 27 (25%) were contaminated with one or more extraneous viruses. Lactic dehydrogenase virus accounted for 23 (85%) of the contaminated specimens, polyoma for 4, MHV for 3, MVM for one, KRV for one and H-1 for one. Multiple viral contamination was present in 6 specimens. Six tumors were screened for LCM virus and 2,005 XC plaque assays were conducted for the detection of leukemia in cell cultures and animal tissues. The contract produced and maintained an inventory of approximately 36 different viral diagnostic reagents. 6,450 ml. of viral reagents and 405 ml. of specific anti-viral antisera were produced. These reagents are available and were supplied on request to NCI investigators. The contract performed diagnostic tests by direct or indirect immunofluorescence on 57 specimens. Direct isolation of viruses from tissues of diseased animals in cell cultures was frequently used particularly when Sendai virus, MHV or LCM virus were suspected.

An important funciton of the contract was to provide support services not available to investigators in other NCI sponsored programs. Some of the projects were: characterization of the gene-dependent expression of murine leukemia; studies on the infectivity of polyoma virus DNA in mice and hamsters; studies on murine C-type viral isolates; and assistance in the diagnosis and control of disease epizootics of suspected viral etiology.

Significance to Biomedical Research and the Program of the Institute:
The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent colonies and laboratory animal-produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems.

Proposed Course: To continue the serodiagnostic services outlined above and to improve the sensitivity of the tests. To apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

NAVY, DEPARTMENT OF (YO1-CP-8-0500)

Title: Development and Characterization of Cell Substrates for Utilization in Cancer Research and Allied Studies

Contractor's Project Directors: Dr. Stewart Madin
Dr. Walter Nelson-Rees

Project Officer (NCI): Dr. Jack Gruber

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biosciences Laboratory (NBL), Oakland, California. This project includes the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karyotyping of cell cultures, and performing biophysical, virological, and cytogenetic applied research.

Major Findings: During the reporting period, the contractor continued to initiate, grow, preserve, characterize, and distribute a variety of human and animal cells for utilization in cancer research. Procedures utilized are such that antibiotic-free cultivation is achieved in the majority of cases. Routine laboratory procedures are such that mycoplasma contamination occurs at only a very low frequency among the numerous cultures initiated, grown, and preserved in this laboratory.

During the six month initial reporting period, 477 cell culture seed stocks (ampoules and flasks) were distributed to 135 individual scientists in 152 shipments. Additionally, 16 pellets and 16 tissue culture fluids were distributed for enzyme analysis. One hundred and ninety-one tumor-derived cell lines previously frozen in the cell bank after brief initial characterization and storage, were re-examined for the presence of unusual non-fibroblastic cell types. Of these, 75 cell lines (40%) were judged to contain significant numbers of either epithelial-like cells or "transformed cancer-like" cells. Selective trypsinization, cloning, and other methods have allowed the CCL to grow pure cultures of cancer cells from five of these mixed cell populations. In the majority of cases, normal fibroblastic cell lines were obtained from the same materials. Further characterization of these cancer cells (and matched normal cells) is in process. Efforts to isolate unusual cell types from the other 70 selected lines will continue.

A number of valuable cell lines of tumor origin have been received from other laboratories and processed at CCL for distribution; among these were four of human origin. Approximately 20 laboratories have submitted 101 individual cell cultures for analysis of species and cell line purity. Of these 96 could be studies. Of these 15% were as purported, three cases were HeLa contaminants, others involved intra- and inter-species contamination. Activities of this nature are projected to continue at the same level for the remainder of the contract year.

Morphologically abnormal cell lines are being compared to morphologically normal cells in terms of growth pattern, cell doubling time, saturation density, clonal growth on various substrates, karyology, and tumorgenicity in nude mice. Clonal growth studies are in progress on a variety of low passage, nonHeLa, human cancer cell lines in an effort to define the optimum nutrition and environmental conditions for culture of such cells.

Techniques for isoenzyme analysis by acrylamide gel electrophoresis have been improved and used to assay for glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconic dehydrogenase (6PGD), and alkaline phosphatase isoenzymes in a variety of cell structures. A commercial preparative-scale electrophoresis apparatus is being investigated with a view to possible performance improvement and its subsequent utilization in the examination of growth factors associated with various tissue culture media components.

Karyologic characterization of all cells maintained in the repository continues and specific collaborative programs requiring the use of karyologic data are in progress.

Significance to Biomedical Research and the Program of the Institute:
The contractor has an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators, to VOP contract laboratories, and the general scientific community. The contract continues to develop techniques for the identification and study of tumor cells oriented toward a study of the fundamental biology of tumor cells and the interaction between tumor cells and viruses of oncogenic importance.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; continue a reference laboratory for karyology of cells in culture; continue repository and distribution functions.

Date Contract Initiated: October 1, 1977

PFIZER, INC. (NO1-CP-3-3234)

Title: Large-Scale Tissue Culture Virus Production for Cancer Research

Contractor's Project Director: Dr. Sami Mayyasi

Project Officer (NCI): Dr. Jack Gruber

Objectives: To provide a service facility for the production of large volumes of selected oncogenic and suspected oncogenic viruses, cellular antigens, tissue culture cell lines, and specific antisera to various oncogenic viruses. Production of these materials is supported by appropriate laboratory groups whose activities include process improvement, product standardization, quality control testing, and applied developmental research.

Major Findings: The current annual rate of large-scale tissue culture production results in over 28,000 liters of virus harvest fluids and over 1,200 grams of cells being processed to fulfill the needs of VOP collaborating investigators. During the past 12 months these materials were distributed in over 400 shipments to approximately 140 laboratories throughout the world. The major viral products generated included: Mason-Pfizer monkey virus (MPMV), which accounted for approximately 20% of the contractor's output; baboon endogenous virus (BeV), 26%; woolly monkey sarcoma virus (SSV-1), 18%; feline leukemia virus (FeLV), 17%; RD-114 virus, 13%; and Epstein-Barr virus. Of all viruses produced, more than 50% were grown on human cells (i.e. NC-37 lymphoblastoid line, A204 rhabdomyosarcoma line). The production of EBV in the P3HR-1 and B95-8 cell lines account for only approximately 1% of the total volume production effort, but the manipulation and concentration of these agents requires a disproportionate amount of time and effort as compared to the RNA type C virus production, and EBV preparation is a significant activity. Additionally, in support of collaborative studies being conducted at the IARC, Lyon, France, slides of concentrated P3HR-1 cells and RAJI cells are being produced and supplied to IARC for EBV epidemiologic titrations.

During this report period a major new effort was included in the workscope. This involved the production and supply to Program of three animal retroviruses with special characteristics. Production concerned preparation of high molecular weight RNA-containing BeV, SSV-1, and FeLV for a specially coordinated molecular epidemiology study. This laboratory helped coordinate the overall virus production effort and the distribution to the collaborating research laboratories.

A major effort was also initiated during this year to modernize the production methodology and improve the quality of product. Roller bottle tissue culture systems were introduced for growth of several of the primate retroviruses, growth of cells on microcarriers is now being studied, and new equipment was purchased to monitor the separation of viruses and viral components in gradients to enhance the purification and concentration procedures. The former tedious procedure for preparing concentrates of infectious EBV will be replaced by simpler procedures as a result of developmental studies on methods to process large volumes of this virus. New facilities to house the DNA

virus production and testing program were constructed by the contractor and recently became operational.

The contractor continued to supply the Program with highly specific reagents including antisera and subviral components. Type-specific radioimmunoassays utilizing low molecular weight proteins of BV and is now employed to distinguish the BV from the RD virus. The low molecular weight proteins of MPMV and SSV-1 have been isolated and type-specific tests for these proteins is under development.

Significance to Biomedical Research and the Program of the Institute: Since its inception, this contract has been a valuable resource for intramural laboratories and collaborating investigators involved in virus-cancer research. The staff and facilities have been consistently responsive to changing needs of NCI. They have provided support to a wide variety of collaborating investigators making possible studies on viruses in cancer that could not otherwise be conducted. Research to determine the association of viruses with human neoplasia have involved activities concerned with molecular biology, biochemistry and immunology. These types of investigations may provide clues to the mechanism whereby viruses mediate the transformation process. Such knowledge could indicate methods by which neoplastic transformation can be averted or inhibited and provide appropriate control measures applicable to the human cancer situation. Studies of this type have created a substantial demand for large quantities of concentrated and purified oncogenic and suspected oncogenic viruses. This contractor has both the capability to help meet such needs and the flexibility to quickly accommodate shifts in Program requirements.

Proposed Course: The production of viruses and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP-7-1014)

Title: Marmoset Colony for Cancer Research

Contractor's Project Director: Dr. Lauren G. Wolfe

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The aim of this contract is the development and maintenance of a marmoset breeding colony in order to ultimately have appropriate numbers of these animals available for experimental use, and to provide a marmoset facility that includes a support laboratory for the inoculation and monitoring of animals under study as well as an adequate animal containment holding area.

Major Findings: The present colony consists of a total of 441 marmosets including 178 breeders, 121 young uninoculated animals and 112 experimental animals. The breeding colony contains 41 cotton-topped, 130 white-lipped and seven common marmosets. During this report period cotton-topped breeding pairs produced the best reproductive performance of the three species. Sixteen term pregnancies yielded 29 live offspring, 20 of which are surviving. Twenty-four white-lipped deliveries yielded 29 live offspring (19 survivors) and four common marmoset deliveries yielded eight live offspring (six survivors).

Clinical laboratory support has been provided for the routine monitoring of the animals and for performing selected techniques designed to improve the reproductive efficiency of the breeding colonies. The clinical service support includes hematology, microbiology, cytology, urinalysis, clinical chemistry, surgery and pathology.

During this report period four special projects were initiated or continued at the request of the NCI Project Officer with the approval of the Program Resources and Logistics Primate Utilization Review Group.

Minor renovations of the facility have permitted implementation of a clean-dirty corridor concept as well as an appropriate flow pattern for personnel, cages and animal wastes.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. The marmoset appears to be especially suitable for use as a comparative model system. To date, at least five and possibly six virus tumor models, including Epstein-Barr and Herpesvirus saimiri viruses, have been established in marmoset monkeys. In addition, because of its small size the marmoset is more economical to house yet it is large enough for routine surgical procedures and serological monitoring.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: April 1, 1977

ST. JOSEPH'S HOSPITAL (NO1-CP-3-3393)

Title: Tissue Procurement from Patients with Malignancies

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: To supply investigators within the Viral Oncology Program with fresh specimens of tissue from human malignancies obtained as a byproduct of necessary surgical procedures.

Major Findings: During the current period, the contractor shipped 160 surgical specimens in culture medium to the NCI resources processing laboratory for rapid delivery to research investigators. These included 83 malignant, 24 benign, and 53 normal tissues. In addition, 43 malignant, 33 benign and 26 normal tissues were collected clean but not sterile and shipped frozen for distribution to laboratories requiring bulk tissues for chemical studies. More than 400 serum or plasma specimens were collected from these patients and from other donors for shipment to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: Availability of clinical specimens and pertinent clinical information on the cases from which they came is essential to research on the possible role of viruses in human neoplasia.

Proposed Course: The contractor will continue the activities described above.

Date Contract Initiated: June 24, 1969

ST. JUDE CHILDREN'S RESEARCH HOSPITAL (NO1-CP-7-1030)

Title: Human Specimen Acquisition for Cancer Research

Contractor's Project Director: Warren Johnson, M.D.

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. Jack Gruber

Objectives: To supply research investigators within the Viral Oncology Program with fresh neoplastic tissue specimens, including solid tissues and blood components and with specimens from other diseases treated at St. Jude Children's Research Hospital.

Major Findings: During the initial months of this new effort the contractor has supplied 98 frozen tissue specimens and three sera acquired from approximately 15 patients. Efforts are currently underway to clarify the histopathological characterization of many of the specimens received.

Significance to Biomedical Research and the Program of the Institute: The availability of fresh clinical specimens would markedly enhance studies on the possible viral etiology of human neoplasia.

Proposed Course: The type of specimens acquired from this source may not be required in the future.

Date Contract Initiated: September 22, 1977

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-3-3340)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. David McB. Howell

Objectives: To breed, maintain, and supply young and mature chimpanzees to investigators for research in the sub-human primate on the possible viral etiology of human cancer.

Major Findings: The existing chimpanzee colony numbers 31 animals including two breeding males, six breeding females, three juvenile males, 11 juvenile females, one infant, and an additional eight infants assigned to six experimental studies initiated by collaborating Viral Oncology Program investigators. During this report period one live birth occurred on October 13, 1977.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. Newborn chimpanzees are particularly useful in determining susceptibility to suspected human cancer viruses because their resistance to virus infection is very low. This is currently the only source of newborn chimpanzees for the VOP.

Proposed Course: The chimpanzee colony will be maintained, and newborn animals will continue to be supplied to investigators within the VOP.

Date Contract Initiated: April 25, 1969

STANFORD UNIVERSITY (NO1-CP-6-1026)

Title: Development of a Method for the Large-Scale Production of DNA Ligase and DNA Polymerase I from *E. coli*

Contractor's Project Directors: Dr. I. R. Lehman
Dr. Arthur Kornberg

Project Officers (NCI): Dr. Edward Scolnick
Dr. Robert Gallo

Objectives: To develop a method for production on a large scale of DNA ligase and DNA polymerase I and to utilize this method to produce these enzymes in quantities from ten to one hundred times greater at any given scale than methods previously available.

Major Findings: The contractor has constructed a hybrid bacteriophage lambda DNA containing the gene for E. coli DNA ligase.

In an effort to further increase the yield of DNA ligase, the contractor has modified the phage lambda DNA molecule to produce a vector which, under the appropriate conditions, generates extremely high levels of DNA ligase. The new vector λgt 4-lop 11 lig+, can form a stable lysogen which upon heat induction produces a 100-fold increase in DNA ligase activity. The introduction of a phage mutation (S7) that prevents cell lysis results in an even greater increase (500-fold) in DNA ligase activity. Under these conditions, the level of DNA ligase in extracts of the induced lysogen is approximately 5% of the total cellular protein of E. coli. Starting with this highly enriched extract, the contractor has developed a simple three-step purification procedure that leads easily to homogenous enzyme in excellent yield.

Application of the techniques that proved successful in cloning the DNA ligase gene were ineffective in the contractor's attempts to clone the gene for E. coli DNA polymerase I. A plausible though unproven explanation for their failure is that either the polA gene contains an EcoRI endonuclease site, or that the sites flanking the polA gene are so widely spaced that the restriction endonuclease fragments containing the polA gene are too large to permit them to be incorporated into a viable lambda DNA particle. Possibly, vectors containing restriction endonuclease sites other than EcoRI, for example HindIII, might serve in cloning of the DNA polymerase I gene.

Significance to Biomedical Research and the Program of the Institute: DNA polymerase I and DNA ligase have been of great value in a variety of studies directly related to cancer. Among these studies is the preparation of probes to determine whether certain RNA and DNA viruses may be involved in human malignancy.

Proposed Course: This effort was terminated on December 21, 1977 at the request of the contractor. They indicated that the cloning of the DNA ligase gene was successful in producing excellent yields of the enzyme, therefore, this aspect of the contract had been satisfactorily concluded. They further indicated that attempts to achieve overproduction of the DNA polymerase I gene were voluntarily terminated.

Date Contract Initiated: December 22, 1975

TENNESSEE, UNIVERSITY OF (NO1-CP-6-1020)

Title: Supply of Fresh and Frozen Brain Tissues for Cancer Research

Contractor's Project Director: Dr. E. M. Stadlan

Project Officer (NCI): Dr. John S. Cole III

Objectives: To supply NCI with fresh, viable, and frozen human brain tissue and brain tumors for subsequent distribution to investigators for cancer research.

Major Findings: The contractor is located in Memphis, Tennessee, which is the center for neurosurgical effort in the surrounding five-state area. A large number of neurosurgical procedures are performed in the participating hospitals, and the principal investigator, a neuropathologist, together with the co-principal investigator, the Chairman of the Neurosurgery Department, have access to all by-product brain material resulting from normal and standard surgical procedures.

During the current period 156 malignant and benign brain tissue specimens were collected. A total of 78 specimens from 76 subjects were shipped to the NCI repository. A total of 78 sterile specimens from 78 subjects were shipped to the NCI resources processing center. Tissue bearing the following diagnoses were shipped: astrocytomas, gliosis, meningiomas, pituitary tumors, acoustic neuromas, gliomas, metastatic tumors, schwannomas.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract of major importance to the NCI, since it is a primary source of diverse brain tumor specimens for NCI researchers. A continuing supply of such specimens is necessary for the pursuit of the viral etiology of human cancer.

Proposed Course: To continue to supply fresh tissues to the Viral Oncology Program.

Date Contract Initiated: January 19, 1976

TULANE UNIVERSITY (NO1-CP-3-3396)

Title: Maintenance of a Colony of Nursery-Reared, Seronegative, HVS-free Squirrel Monkeys

Contractor's Project Director: Dr. S. R. S. Rangan

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The maintenance and monitoring of a colony of Herpesvirus saimiri (HVS)-free, seronegative squirrel monkeys in order to have appropriate numbers of these animals available for experimental use.

Major Findings: The colony of HVS-free, seronegative squirrel monkeys was developed over a three year period as part of a multifaceted research contract effort. In June 1976 the research portion of the multifaceted contract was terminated and the animals became a Program resource. Currently the colony consists of a total of 39 animals (16 males and 23 females) ranging in age from 2 1/2 to 4 3/4 years of age. During this report period five births - one stillborn and four live - occurred among the animals born in 1973 and 1974. These animals are housed in a specially fabricated facility far removed from the other squirrel monkey colonies. Admittance to these facilities is restricted to authorized animal care attendants and professional staff required for the maintenance of the colony. Personnel entering the seronegative squirrel monkey colony must adhere to strict clothing requirements and at no time handle or care for squirrel monkeys in the other HVS-seropositive squirrel monkey colonies. Periodic serological and virological monitoring of the animals indicated that they are free of HVS infection. Behavioral observations of the mother and other colony members towards the infants did not indicate any atypical behavioral patterns.

Significance to Biomedical Research and the Program of the Institute: This colony of seronegative, HVS-free squirrel monkeys provides a unique opportunity to evaluate the age-dependent host factors in HVS oncogenesis in the natural host, as HVS is endogenous in squirrel monkeys with the majority of feral and colony-reared animals possessing antibodies to the virus. Because the HVS-squirrel monkey animal model may resemble the relationship of EBV and Herpes simplex virus in humans, information gleaned from studies of HVS in seronegative squirrel monkeys may possibly be applicable to the human situation.

Proposed Course: The seronegative squirrel monkey colony will be maintained, and a breeding effort will be continued with the sexually mature animals in the colony.

Date Contract Initiated: June 29, 1976

UNIVERSITY HOSPITALS OF CLEVELAND (NO1-CP-6-1007)

Title: Supply of Pediatric Specimen Material from Neoplastic Diseases

Contractor's Project Directors: Dr. Samuel Gross
Dr. Robert J. Izant, Jr.

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: To procure whole blood, bone marrow, and tissues from pediatric subjects with neoplasms for use by collaborative cancer research investigators.

Major Findings: The active organization for this contract within the University Hospitals of Cleveland is the Department of Pediatrics at Rainbow Babies and Childrens Hospital. The contractor is a primary, secondary, and tertiary hospital consisting of 97 surgical and 123 medical beds. The principal investigators have access to all pediatric material; during the current period, a total of 152 specimens were shipped to NCI resources processing center and to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: The difference in the spectrum of tumors seen in childhood along with some apparent differences in their natural history and response to therapy make pediatric neoplasms an especially interesting class of tumors to study from a virologic, biochemical, and immunologic point of view.

Proposed Course: With the completion of scheduled activities, this contract will terminate on October 14, 1978.

Date Contract Initiated: October 15, 1975

UNIVERSITY LABORATORIES, INC. (NOI-CP-3-3222)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Dr. Garrett V. Keefer
Dr. John S. Cole III

Objectives: To supply a variety of oncogenic viruses and their antisera in volumes necessary to meet VOP research needs.

Major Findings: The contractor's production of Rous sarcoma virus (Prague strain) in tissue culture roller bottles has continued at a very high level. During this report period, approximately 2,677 liters of virus-containing tissue culture fluid were produced; this material was pelleted, resuspended, and issued to various collaborating laboratories at approximately 132X of its original concentration. Production lots were sent to investigators in the U.S. and throughout the world. Recipients included Drs. Baltimore, Bishop, Joklik, Smith, Zhdanov, Cardiff, Faras and others.

During this report period a total of approximately 7,295 ml. of BK virus and 4,297 ml. of BK lysate has been shipped to NCI investigators for developmental research studies.

The production of Moloney sarcoma virus was initiated in March 1978 with 597 ml. of the virus produced during this report period.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses produced by this contractor has been extensively used by VOP researchers and is essential to the continuation of many important research projects presently being carried out in the Program.

Proposed Course: Production of needed strains of oncogenic viruses will continue in volumes necessary to meet VOP needs.

Date Contract Initiated: June 4, 1962

C. 3. PROGRAM MANAGEMENT
a. Contract Reports

HAZLETON LABORATORIES, INC. (NO1-CP-6-1024)

Title: Support Services to Maintain Studies on the Role of Viruses in Experimental Oncogenesis and Human Cancer

Contractor's Project Director: Dr. David Valerio

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: The objective of this contract is to provide services in support of research into the role of viruses in experimental oncogenesis and human cancer.

Major Findings: During the past year, this project provided essential services to support in-house research of the Molecular Biology Section, Laboratory of RNA Tumor Viruses. The contractor prepared tissue culture medium according to rigid standards for sterility and cell viability and provided freezer space for storage of culture medium. The project produced special viruses and cells in large quantities for NCI staff and established and propagated human tumor cell lines and other special lines in tissue culture as reagents for the Viral Oncology Program.

The contractor also provided standardized assays according to established protocols for the detection and quantitation of type C viruses in samples supplied on a routine basis by NCI staff. These tests included radioimmunoassays for type C viral polypeptides, the reverse transcriptase assay, RNA-DNA and DNA-DNA hybridization, and biologic assays including the focus forming assay for sarcoma viruses and the X-C plague assay for leukemia viruses. Electron microscopy was also available as a service on a limited basis.

Other services provided by the project in support of NCI in-house research included glassware facilities and care and holding of animals. The project provided facilities which strictly adhered to biohazard regulations of NCI. These included separate laboratories for work with different viruses, including those classified as moderate risk agents.

Significance to Biomedical Research and the Program of the Institute:

This project provides essential functions for highly productive in-house research whose goals are to establish a viral etiology of human cancer and to develop methods by which to prevent this disease. The project also provides the Viral Oncology Program important resources including human tumor cell lines established in tissue culture, specialized viruses, and testing services for detection and identification of new virus isolates.

Proposed Course: The contractor has proven expertise in providing these services and has a very high probability of continuing these efforts at a superior level of performance.

Date Contract Initiated: September 1, 1972

LITTON BIONETICS, INC. (NO1-CP-4-3249)

Title: Support Services for the Application of Animal Virus Model Systems to Human Neoplasia

Contractor's Project Director: Dr. P. Weislogel

Project Officer (NCI): Dr. Robert Bassin

Objectives: the purpose of this project is to provide facilities and staff to support laboratory research investigations by intramural NCI scientists.

Major Findings: During the past 12 months the contract has supported NCI research investigators by the propagation and maintenance of approximately 100 cell lines, each in a well-defined, stable condition as required for particular experimental uses. It has performed *in vitro* infectivity assays for murine sarcoma and leukemia viruses and has also carried out assays for DNA polymerases in virus and cell preparations, as well as complement fixation and radioimmunoassay tests for virus-related antigens.

Other support has included the production, characterization, and storage of new cell lines; production and characterization of virus stocks; fractionation of cell extracts for studies on virus-associated enzymes; and production and characterization of immune sera and immunocompetent cells.

Significance to Biomedical Research and the Program of the Institute: An understanding of viral defectiveness and the role "helper" viruses play is of value in determining the occurrence and mechanism of viral oncogenesis by type C viruses in man. The identification of viruses or viral products in human tumor cells is of value in assessing the role of viruses in human cancers, and ultimately, in developing techniques of both diagnostic and therapeutic significance. The contract provides the support facilities necessary for the carrying out of this research.

Proposed Course: Since space on the NIH reservation became available to conduct this research, this contract was terminated on July 31, 1978 and Dr. Bassin returned to NIH.

Date Contract Initiated: June 27, 1969

MELOY LABORATORIES (NO1-CP-4-3207)

Title: Support Services for Studies on Spontaneous and Virus Induced Neoplastic Transformation

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. George J. Todaro

Objectives: The objective of this contract is to provide facilities and support services for intramural studies on spontaneous and virus-induced neoplastic transformation.

Major Findings: During the past year this contract provided necessary facilities and services to support in-house research of elements of the Laboratory of Viral Carcinogenesis, VOP, DCCP, into the viral etiology of human cancer. Among the services furnished by the contractor were: a) preparation and formulation of tissue culture media; b) maintenance of human and non-human cell lines; c) large-scale production of cells; d) initiation of new cell lines; e) maintenance of animal facilities; f) maintenance of stocks of oncogenic and potentially oncogenic human and animal viruses; g) routine serologic and biochemical assays for viruses; h) routine nucleic acid hybridizations; i) immunization of animals; j) routine immunofluorescent assays; k) electron microscopy l) histology and pathology; and m) glassware services.

Significance to Biomedical Research and the Program of the Institute: Important findings regarding the mechanism of action of oncogneic viruses, their effects on cellular growth control mechanisms and their possible involvement in natural oncogenesis have been developed from a number of projects on this contract. A number of new type C viruses have been isolated and characterized and have provided much information on the nature of type C virus-cell interactions. Hopefully, the techniques employed in inducing and growing these viruses may be used to isolate a complete human type C ivrus which would immeasurably aid in studies of the possible viral involvement in human cancer. The services afforded by this contract are of essential importance in furthering this research.

Proposed Course: To continue to provide services and facilities in support of the indicated research.

Date Contract Initiated: May 25, 1965

MELOY LABORATORIES (NO1-CP-4-3223)

Title: Support Services for Molecular Studies of Human and Animal Cancer with Emphasis on Mammary Carcinoma

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: The objective of this contract is to provide support services and facilities for molecular and immunologic studies of human and animal cancer with emphasis on mammary carcinoma.

Major Findings: During the past year, this contract provided necessary facilities and services to support in-house research of elements of the Laboratory of Viral Carcinogenesis, VOP, DCCP, concerning the viral etiology of cancer in experimental animals and humans. Among the services furnished by the contractor were: preparation and formulation of tissue culture media; maintenance of human and non-human cell lines; large scale production of cells; initiation of new cell lines; maintenance of animal facilities; maintenance of stocks of oncogenic and potentially oncogenic primate and rodent viruses; routine biochemical assays for viruses; routine nucleic acid hybridizations; immunization of animals; electron microscopy; glassware services; and preparation of viral structural proteins and enzymes used to study RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The ability to detect viral markers in human tissues is basic in the establishment of an etiologic relationship between viruses and cancer and may prove extremely useful in the early detection and treatment of certain forms of cancer. This contract provides services and facilities essential to studies whose purpose is to develop this ability.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: July 20, 1973

MELOY LABORATORIES (NO1-CP-4-3236)

Title: Support Services for Immunological and Biochemical Studies of Mammalian Viral Oncology

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Edward Scolnick

Objectives: The purpose of this contract is to provide support services and facilities for studies on the immunology and biochemistry of mammalian viral oncology.

Major Findings: During the past year this contract provided essential services and facilities to support in-house research of the Laboratory of Tumor Virus Genetics, VOP, DCCP, concerning the viral etiology of human cancer. Among the services furnished by the contractor were: preparation and formulation of tissue culture media; maintenance of human and non-human cell lines; large-scale production of cells; initiation of new cell lines; maintenance of animal facilities; maintenance of stocks of human and animal oncornaviruses; routine biochemical assays for viruses; routine nucleic acid hybridizations; immunization of animals; routine immunofluorescent assays; electron microscopy; glassware services; and preparation of viral structural proteins and enzymes and cellular enzymes used for the study of RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The ability to detect viral information in transformed cells is basic to establishment of etiological association and to an ultimate approach to prevention or treatment of cancer. This contract provides services and facilities essential to studies whose purpose is to develop this ability.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: May 25, 1965

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-4-3254)

Title: Support Services to Maintain Studies of Type C RNA Tumor Viruses

Contractor's Project Director: Dr. P. Reddy

Project Officer (NCI): Dr. Padman Sarma

Objectives: To provide support services for type C RNA tumor virus studies carried out by NCI personnel or under NCI direction at the contract facility.

Major Findings: The scope of the work was limited to 17 specifically defined service functions itemized under two broadly divided tasks: (1) Virology, and (2) Immunology and Molecular Biology. Services provided under these tasks include isolation of type C viruses; preparation of tissue cultures (primary and established lines); infectivity and other assays for type C viruses; purification of viruses; storage and retrieval of cell cultures and viruses at ultra low temperatures; maintenance of cell repository; determination of infectivity and host range studies using various target cells; procurement and establishment of tumor tissues in culture as designated by the Project Officer; animal inoculation; maintenance of animal facility; collection and testing of sera for sero-epidemiologic survey; preparation of tissues and tissue culture antigens for immunologic procedures; production of high titer monospecific virus neutralizing antibodies; performance of immunologic tests such as immunodiffusion, complement fixation, and immunofluorescence for virus detection;

preparation of antigens and antibodies for radioimmunoassays; preparation of reagents for molecular hybridization with DNA and RNA probes of selected viruses and cellular DNA's; carry out reverse transcriptase assays for the detection of viruses using standard dT.rA template.

Significance to Biomedical Research and the Program of the Institute: The studies supported by the services of this contract provide further information on the natural occurrence and spread of oncogenic type C viruses in homologous and heterologous species, including man.

Proposed Course: Since space on the NIH reservation became available to conduct this research, Dr. Sarma returned to NIH in the summer of 1978. This contract will terminate on December 23, 1978.

Date Contract Initiated: October 23, 1973

LITTON BIONETICS, INC. (NO1-CO-75380), BETHESDA, MARYLAND

Title: Operation and Maintenance of the Frederick Cancer Research Center (FCRC), National Cancer Institute, Fort Detrick, Frederick, Maryland, 21701

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Viral Oncology Coordinator (NCI): Dr. Henry J. Hearn

Objectives:

A. To conduct primary structure analysis of viral and viral related proteins for the entire FCRC/Viral Oncology Program, concentrating on gag and env gene products of various retroviruses; to provide information applicable to the synthesis of specific peptides for improved immunoassays, functional studies, and potential synthetic vaccines; to develop micro-techniques for structural analysis procedures; studies also include transformation and other specific proteins of selected herpesviruses.

B. To develop and apply specific immunoassays to natural history studies of oncornaviruses including surveys of human materials for oncornavirus markers and the isolation and characterization of the feline oncornavirus cell membrane antigen (FOCMA).

C. To conduct studies on the mechanism of induction and repression of type C virus expression, including effort with human cells; to isolate and characterize retrovirus DNA-binding proteins.

D. Investigate control of expression of mouse mammary tumor virus and the immunological response of mice to this agent; to determine constituent viral polypeptides such as precursors to env and gag, and the appearance of these products in the cell cycle and intracellular distribution; develop immuno-assays to evaluate humoral and cell-mediated immunity in the mouse; apply approaches to studies on Mason-Pfizer monkey virus and squirrel monkey retrovirus.

E. Study distribution and expression of viral nucleic acids in various experimental situations, involving selected type C viruses, and their relationship to disease; develop probes specific for such viruses and the detection of the arrangement of endogenous sequences in the host genome.

F. Conduct studies on the isolation and inter-relationships among a number of EBV-related primate viruses; investigate host-virus relationships involving a "factor" produced by H. saimiri transformed cells and tumors that inhibit immune proliferative responses, including purification and characterization of the "factor" together with developing techniques to conduct surveys for its presence in animal models and man.

G. To conduct a major resource effort and provide facilities for the large-scale production of viruses or viral components for use within the FCRC/VO Program, and for outside viral oncology programs as distributed through the NCI's Office of Program Resources and Logistics by providing high quality retrovirus concentrates and related products, by conducting developmental research for determining maximal virus or subviral component expression and recovery and by pursuing product improvement studies.

H. To provide high quality electron microscopic support for the FCRC/VO Program in the form of particle counts and thin section screening of production cell lines; also to make visual determinations of viral RNA structure and apply immunoelectron microscopic methodology to studies on viral proteins.

I. To provide quality control assays for the detection of microbial contamination of all cell culture systems in use throughout the FCRC/VO Program, particularly for the Viral Resources Laboratory.

J. To provide necessary immunology and molecular biology support for an ongoing collaborative program with NCI personnel investigating inter-relationships among type C virus activation and normal host physiological processes, as possible mechanisms by which viruses influence or can be influenced by host-tumor interactions. Results of this work appear elsewhere in the annual report (Dr. Alfred Hellman).

K. To provide necessary support for collaborative studies with NCI investigators on the mechanism of cell transformation by herpesviruses and their possible involvement as co-carcinogens with other viruses or chemicals, and for studies on alterations that occur in transformed cells, focusing on cellular enzymes, processes that repress or derepress normal cell components, and changes in properties of cell membranes induced by virus infection. Results of this work appear elsewhere in the annual report (Drs. Berge Hampar and Masakazu Hatanaka).

L. To provide necessary professional and technical support for a collaborative program with NCI personnel to develop appropriate genetically defined animal model systems for studying the influence of endogenous virus expression on normal cell differentiation and on abnormal changes leading to malignant transformation, to quantitate and analyze translational products of type C isolates, and genetically map selected type C RNA viral genomes. Results of this work appear elsewhere in the annual report (Dr. John Stephenson).

M. To provide necessary professional and technical support for a collaborative program with NCI personnel in evaluating various vaccines and vaccination protocols for their efficacy in preventing the expression of spontaneous, and chemically- or X-ray-induced neoplasia in animal test systems showing low to high expression of endogenous viruses. Results of this work appear elsewhere in the annual report (Dr. Robert Huebner).

N. To conduct a comprehensive safety and environmental control program for the Frederick Cancer Research Center and to perform applied and basic studies and literature surveys for risk assessment in support of the various FCRC operations.

O. To operate an animal farm for the breeding of laboratory animals to meet the needs of research programs at FCRC, and for shipment to other NCI operations as production permits.

P. To provide an Animal Health Diagnostic Section for monitoring the health and genetic quality research animals at the FCRC and NCI in order to ensure the validity of animal-related research and to preclude the entry of undesirable pathogenic microbes, latent murine viruses and parasites.

Major Findings:

Those areas of Contractor operations that were completely funded by Viral Oncology, DCCP, NCI are the following:

Immunochemistry Laboratory: The NH_2-terminal amino acid sequence of p30 of the xenotropic mouse virus, BALB-2, was found to be the same as the sequences of p30s from mouse ecotropic viruses (previous analyses).

The amino- and carboxyl-terminal amino acid sequences of R-MuLV, AKR-MuLV, and M-MuLV gag gene-coded proteins (p10, p12, p15, and p30) were determined. The p15 protein from all three viruses appears to have a blocked amino end. Proline was found to be the common NH_2-terminus of p30s and p12s, and alanine of p10s. The amino-terminal sequences of p30s are identical, as are those of p10s (except a single substitution in M-MuLV proteins); the p12 sequences are clearly distinctive but also show substantial homology. The carboxyl-terminal amino acids of all three viral p30s and p12 are leucine and phenylalanine, respectively. R-MuLV p15 has tyrosine as the carboxyl-terminus, whereas AKR p15 has phenylalanine in this position. The compositional and sequence data provide definite chemical criteria for the identification of analogous gag gene products and for the comparison of viral proteins isolated in different laboratories. On the basis of amino acid sequences and the previously proposed H-p15-p12-p30-p10-COOH peptide sequence in the precursor polyprotein, a model for cleavage sites involved in the post-translational processing of the gag gene coded precursor has been proposed. The cleavage sites based on this model are tyrosylphenylalanine between p15 and p12, phenylalanylproline between p12 and p30, and leucylalanine between p30 and p10. This may indicate the involvement of enzymes with two distinct specificities.

Proline was found to be the common NH_2-terminus of the major internal proteins (p30 homologs) of all retroviruses so far analyzed, including type C, type B (MMTV), type D (MPMV), BoLV, and visna virus. This, together with the similarity of available short sequences, may indicate conservation of a progenitor cleavage site utilized in the generation of the component polypeptides from the gag gene precursor polyproteins. Based on this finding, further sequence homology (not revealed by immunologic techniques) of these proteins of retroviruses is suspected.

Chemical analysis of the gag gene products of highly related type C viruses of primate origin, M-7 (an endogenous baboon virus) and RD-114 (an endogenous feline virus), revealed that both p12 proteins have a blocked NH_2-terminus. RD-114 p15 has a proline NH_2-terminal endgroup and both M-7 and RD-114 p15s have a phenylalanine COOH-terminus. Based on this data and the results of sequence analyses of M-7 and RD-114 p30s (previous findings), the cleavage site between p15 and p30 in both M-7 and RD-114 gag precursors appears to be similar to that postulated for mouse viruses.

Since phenylalaninylproline and/or tyrosylproline linkages (the proposed cleavage sites) are known to be rather resistant to known proteolytic enzymes, it is suspected that a novel enzyme (possibly virus-specific) is involved in gag precursor processing.

Cyanogen bromide cleavage fragments and several chymotryptic peptides from R-MuLV p30 have been purified and subjected to compositional and amino acid sequence analyses. In total, of approximately 270 p30 residues, 25% have been placed in sequence. Immunochemical studies, including radioimmunoassays, have shown that the amino acid sequence delineating the major group-specific antigenic sites of p30 is apparently contained within the 10,000 molecular weight COOH-terminal fragment of the molecule.

Immunochemical and chemical analyses (including amino acid composition and sequence analyses of fragments(provided convincing evidence that the two glycoproteins of gp70 and gp45 of R-MuLV are highly related. It was found that gp70 and gp45 have common NH_2-terminal amino acid sequences, the protein component of gp45 is smaller than that of gp70, and the two glycoproteins have different COOH-terminal amino acids (tyrosine for gp70 and leucine for gp45). These results provided sufficient evidence to suggest that the two glycoproteins are not separately encoded in the viral genome and that gp45, which also contains less carbohydrate, is derived from gp70 through proteolytic cleavage. Cyanogen bromide cleavage of R-MuLV gp70 resulted in the production of three peptide (glycopeptide) fragments which have been purified and analyzed. Amino acid sequence analyses of uncleaved gp70 and its fragments permitted us to place approximately one-fourth of the total amino acid residues of gp70 in sequence. Immunochemical studies have shown two fragments to remain antigenic. Further delineation of immunological sequence determinants is in progress and expected to lead to the production of synthetic vaccines.

Additionally, statements summarizing results of other work are given as follows: Monospecific antiserum to R-MuLV p15(E) has been prepared and the surface localization of this viral protein and that of gp70 has been confirmed by the virolysis assay. Host antigens, including H-2 antigenic determinants, were found by the virolysis assay to be incorporated in the viral envelope of R-MuLV. Several peptides representing the constant and variable regions of type C virus p30s have been synthesized and are being studied for antigenicity. Microtechniques for peptide purification required for subsequent microsequencing have been developed. Novel procedures for viral protein purification have been developed. A previously uncharacterized highly basic polypeptide was isolated from R-MuLV. Techniques for comparative primary structure analyses of viral proteins and glycoproteins have been improved for routine work. Tryptic fingerprint analyses of MMTV p27 and p14 showed that both proteins are derived from and have common peptides with a precursor polyprotein, Pr67. Similar analysis revealed that the envelope glycoproteins gp52 and gp36 have distinct polypeptide moieties and are derived from a common glycosylated precursor, gPr75. Gag precursor polyproteins, Pr67s, of LCV (from L929 cells) and R-MuLV (from virus) have been purified for chemical analyses. The two polyproteins were found to be compositionally and immunologically distinct. The cleavage of Pr67 in L929 cells was shown to be retarded, leading to intracellular accumulation of this precursor. Tryptic fingerprint analyses of immune precipitates from R-MuLV producing cells showed the structural relatedness of gag polyproteins Pr67, Pr80, and a glycosylated product, gPr95. The latter may represent either glycosylated Pr67 or Pr80. Chemical and immunochemical studies have been initiated to localize and characterize the additional peptide sequence in Pr80.

Finally, in oncornavirus immunobiology the Contractor has: Developed a radioimmunoassay (RIA) for the FeLV gp70 and used this to characterize feline sera, particularly from viremic cats. Localized by IEM the staining of FOCMA antibody containing antisera (in collaboration with M. Gonda, Electron Microscopy Group) to both free and budding virions as well as sites on the cell membrane not related to the virus budding. Isolated FL-74 cell membranes using several techniques including the Stansted cell disrupter and the Scott membrane vesiculation procedure. Determined that most feline sera with FOCMA antibodies, even sera from viremic cats with high titered responses, do not efficiently precipitate a specific protein from metabolically labeled FL-74 of FSV-transformed non-virus-producing cells. Performed immune precipitations on FL-74 cells surface labeled with ^{125}I using lactoperoxidase. Presumptive evidence for the surface expression of gp70 and a high molecular weight (85,000 dalton) envelope precursor was obtained; cell surface p30 (presumably a gag precursor), however was not detected. Isolated EIAV p25 and found that virtually all infected horses

make antibodies to this protein in such high titer that ID suffices to make the diagnosis. Additionally, no immunologic cross-reactions were detected either by direct precipitation of competition RIA with various non-type C or type C viruses. Obtained radiochemically pure EIAV p10. Since this protein does not appear to be a target of the natural immune response it should be useful in studying EIAV expression in infected horses. Found that R-MuLV gp45 and gp70 are highly related but, nevertheless, distinguishable by RIA. Identified by iodo-lectin staining a 20,000 dalton glycoprotein in primate derived or associated retroviruses. Isolated SD-RaLV p30, p12, and p15 and developed sensitive RIAs which will be used to study viral protein expression in spontaneous and chemically induced rat neoplasms.

RNA Virus Laboratory: Accomplishments related to the Biology of type B viruses include: Purified and prepared monospecific antisera to gp52, gp36, p27, and p10. Established radioimmunoassays for the characterization of the MMTV DNA-binding protein, p14, and the major envelope glycoprotein gp52.

Studies on MMTV gene expression, protein synthesis, processing, and cell distribution have demonstrated that: MMTV glycoproteins and nonglycoproteins are synthesized via separate polypeptide chain initiation sites and, therefore, are not synthesized as one large precursor polyprotein. MMTV glycoproteins gp52 and gp36 are distinct protein components derived from a glycosylated precursor polyprotein of 75,000 daltons which is a primary translation product, gPr75, and contains only gp52 and gp36 (gPr75-MMTV env). The gene order for gPr75 is: (5')gp52-gp36(3'). MMTV nonglycoproteins are derived from a common precursor polyprotein of 75,000 daltons which binds to ssDNA (Pr75-MMTV gag). Pr75 contains at least p27, p14, and p10. Noncoordinate expression of gPr75 and Pr75, i.e., the synthesis of MMTV glycoproteins, is not contingent upon the concurrent synthesis of MMTV nonglycoproteins. GPr75 and Pr75 are synthesized sequentially (gPr75 followed by Pr75) and between late G_2 and early G_1. No MMTV precursor polyproteins or labeled gp36 are present on the cell surface, indicating that gPr75 (MMTV env) is cleaved and the glycoproteins properly oriented prior to appearance of gp52 on the cell surface. The following cultured mammary tumor cells contained both gp52 and gp70 as cell surface antigens: cells producing only MMTV, cells producing both MMTV and MuLV (these also exhibited GCSA-like surface antigens), and non-virus-producing cells synthesizing high levels of MMTV antigens. Mammary tumor cells from C3H mice never placed in culture possessed only gp52 and gp70 as viral cell surface antigens; lymphocytes from the same animals, as well as GR/N and C67BL/6 mice contained only gp70. The MuLV produced by the mammary tumor cells is an amphotropic virus possessing both ecotropic (N- and B-tropic) and xenotropic properties. It is the first amphotropic MuLV from a C3H mouse and is also the first derived from a cell of mammary tumor origin.

In understanding the nature of immune recognition of MMTV expression it was found that: Complement-dependent serum cytotoxicity and membrane

immunofluorescence assays using monospecific antisera detected MMTV gp52 and MuLV gp70 as cell surface antigens on mammary tumor cells. Gp52 and gp70, detected by cytotoxicity, represent membrane antigens which are integral components of the cell surface, because they are detected on cells not producing MMTV or MuLV. Procedures such as trypsinization or versenation expose gp36 and p10, but no p27 on the membranes of mammary tumor cells. Strains of mice which express MMTV develop antibodies which are specifically cytotoxic for mammary tumor cells producing MMTV. The distribution of gp52 in mouse tissues was predominantly found in organs with secretory functions such as mammary tissue of C3H/HeN, C3H/HeNf, and GR/N females as well as the vas deferens, seminal vesicles, and submaxillary glands of C3H/HeN, C3H/HeNf, and GR/N males. Gp52 and natural antibodies which precipitate MMTV co-exist in the sera of tumor-bearing mice. The antigen exists predominantly as a free protein and the antibodies are found in the 7S immunoglobulin fraction. Autogenous immunity develops against the endogenous MMTV since precipitating antibodies can be demonstrated in both male and female C3H/HeNf and BALB/c NIV mice. Antibodies against purified gp52 have been found in the sera of mice which have high titers against intact MMTV. Feral mice, which have a low spontaneous mammary tumor incidence, express MMTV gp52 and precipitating antibody to MMTV but at lower levels and frequencies than observed in inbred mice of high and low mammary tumor strains. Spleen lymphocytes from strains of mice which express MMTV but do not have mammary tumors respond in a blastogenesis assay to MMTV and MuLV, and the lymphocytes can be stimulated with gp52 and gp70. This response appears to be age-dependent.

In attempting to alter natural immune responses in mice it was found that: Mice immunized with MMTV develop both cytotoxic antibodies to MMTV-producing cells and precipitating antibody predominantly directed against the envelope proteins gp52 and gp36. C3H and C3Hf mice differ in their response to tumor induction with a clone of Mm5mt/c_1 cells which produces high levels of MMTV. The C3Hf mice appear resistant as compared to C3H and athymic mice, suggesting an immunologic mechanism for the resistance.

In examining the distribution of proviral sequences and expression of type D viruses in various primates it was found that: Approximately 20% of the MPMV genome is present in the cellular DNA of several Old World monkeys of the subfamily Cercopithecinae (rhesus, baboon etc.). SMRV is an endogenous virus of squirrel monkey, a New World primate, and is represented in cellular DNA in multiple copies (5-10). SMRV 70S RNA probes show no significant hybridization to DNAs of other New World monkeys, Old World monkeys, and apes, including man.

In characterizing the structural proteins of MPMV and SMRV it was found that: SMRV contained four major polypeptides with molecular weights of 35-40,000 (p35), 20,000 (p20), 14,000 (p14), and 8,000 (p8) daltons regardless of whether the virus was propagated in human, mink, or dog cells, indicating that these four proteins are virus-coded. Human- and mink-grown SMRV

contain a 73,000 dalton glycoprotein, whereas canine-grown SMRV contains a 100,000 dalton glycoprotein. Although MPMV and SMRV show no immunological cross-reactivity in homologous RIAs for the major nonglycoprotein of each virus, interspecies relatedness based on the results of an interspecies radioimmunoassay using MPMV p27 antiserum and ^{125}I-SMRV p35 was demonstrated. Using group, type, and interspecies radioimmunoassays for MPMV p27 and gp70, the X381 and FTP-1 virus isolates were indistinguishable from MPMV.

In understanding the nature of immune recognition of type D virus expression it was found that: MPMV-inoculated rhesus monkeys and monkeys housed with MPMV-infected monkeys developed high-titered antibodies which precipitate intact MPMV as well as purified MPMV p27 antigen. These antibodies persist throughout the animals' lives. Natural antibodies cross-reactive with MPMV were found in many of the imported and uninoculated caged rhesus monkeys. Natural antibodies cross-reactive with both MPMV and SMRV in addition to BaEV were found in many of the same rhesus monkeys using a radiolabeled intact virus assay. Cross-absorption studies demonstrated that these antibodies were reactive against common antigenic determinants shared by these viruses. This formed the basis for another interspecies radioimmunoassay. Natural antibodies were also detected in squirrel monkeys against SMRV and SMRV p35, suggesting that these animals developed an immune response against their endogenous virus. Peripheral blood lymphocytes from rhesus and squirrel monkeys respond in a blastogenesis assay to MPMV and SMRV. Some monkeys respond to both MPMV and SMRV, suggesting cross-reactivity between these viruses.

Accomplishments related to the Biology of type C viruses include: identification and purification to homogeneity of DNA-binding proteins from retroviruses; isolation of two proteins (130,000 and 12,000 daltons) from H. saimiri nucleocapsids that bind to double-stranded DNA; amino acid analogs incorporated into protein (canavanine) efficiently induce type C virus from mouse cells; amino acid alcohols which deplete cells of single amino acids efficiently and reversibly induce type C virus and block protein synthesis in mouse cells; and inhibitors of proteolysis can block induction of type C virus in mouse cells.

In studies on the molecular biology of oncornaviruses closed circular proviral DNA was detected in the nucleus of mouse cells 2.5 hours after infection with R-MuLV. Strong stop cDNA synthesized in the endogenous viral polymerase reaction of R-MuLV, which represents the 5'-terminal region of the viral RNA, was purified, and its partial sequence was determined. An initiator codon was found in this region which thus might specify the NH_2-terminal region of the gag gene precursor polyprotein. Virus-specific circular DNA was detected free in the nucleus of Balb/c cells after cycloheximide induction. Hybridization studies of equine infectious anemia virus (EIAV) with ^{3}H-cDNA prepared in the endogenous reverse transcriptase

reaction showed that virus-infected horse cells, but not normal cells, contained proviral DNA. No sequences related to EIAV were detected in the cellular DNA of other mammals or in the viral RNA of other retroviruses. Reaction products of EIAV reverse transcriptase were found to consist of two distinct classes of DNA, the larger one being 4,000 nucleotides long and the smaller one 500. They were found to represent viral (-) strands and (+) strands, respectively. Very recently, conditions for synthesis of full-length (-) strand cDNA have been found and preparation of a map of the viral genome using restriction endonucleases has begun. The cellular DNA of the natural host of the endogenous baboon virus M-7, Papio cynocephalus, contained about 100 copies of the integrated proviral DNA per haploid genome, whereas virus-producing heterologous host cells (human or dog) contained three to seven copies. The latter DNA, but not baboon cell DNA from non-producing cells, was infectious for permissive cells.

FeLV- and RD-114-related DNA sequences were fractionated into the intermediary repeated sequences of cat cell DNA, confirming the presence of multiple copies of these proviral DNAs in cat cells. Despite the presence of about 20 copies of RD-114 viral DNA in all cat cells, infectious RD-114 DNA was obtained only from virus-producing cat cells. A portion of the FeLV-genome was found only in virus-infected cell DNA. This was detected by FeLV ^{3}H-cDNAs which were pre-absorbed with normal cat cell DNA to remove those sequences related to FeLV found in all cat cells. The multiple copies of FeLV-related proviral DNA appear to be arranged identically in cat chromosomal DNA. This was revealed by the cleavage patterns of various cat cell DNA obtained using various endonucleases followed by in situ hybridization. High molecular weight viral RNA of the Richard and Theilen strains of FeLV were shown to consist of two and three distinctive types with respect to their shape and length based on electron microscopic observation.

Various non-producer clones of SSV-1 transformed rat cells with differential expression of viral gag genes were studied with respect to their content of viral information. There was no direct correlation between the extent of gag gene expression, the magnitude of viral RNA transcription, or the major size class of cytoplasmic viral RNA.

DNA Virus Laboratory: Accomplishments in this area include isolation and initial characterization of H. pongo, its cell lines of origin, and its comparison to other B-cell tropic herpesviruses, serological comparisons of major antigen complexes of EBV-like simian viruses, development of methods for demonstration of specific CMI responses to owl monkeys thereby allowing similar assays for virus-associated antigens to proceed, and first evidence that experimental EBV-induced tumors in marmosets are uniclonal.

Research was conducted in several areas relative to primate lymphotropic herpesviruses. A program on cocarcinogenesis involving Epstein-Barr virus (EBV), various oncornaviruses, and chemical mutagens was initiated in

collaboration with Dr. B. Hampar (Biology of DNA Tumor Viruses, NCI/FCRC). During the past year another member of the EBV group of B-cell tropic primate viruses was isolated from an organutan lymphoid cell line. Other members of this virus group have been isolated from baboons and chimpanzees. A series of studies designed to compare these viruses antigenically, biochemically, and biologically was undertaken. Work on these viruses has extended to seroepidemiologic areas concerning the relationship of different members of this group to lymphoid disease in baboons, to Burkitt's lymphoma (BL) and nasal pharyngeal carcinoma (NPC) in humans, and to possible connections with autoimmune disease in humans. Investigations of various aspects of the immunology of the Herpesvirus saimiri-induced T-cell lymphoma in owl monkeys, emphasizing investigation of cell-mediated immune (CMI) reactions and further studies of the antiproliferation factor (APF) produced by H. saimiri-tumor were continued. These latter studies were designed to explore the effects of APF on various types of immunological functions and to purify and chemically characterize it. In its role as a member of the Herpesvirus Advisory Team on Nonhuman Primate Viruses of the World Health Organization, this laboratory took part in a study of the possible infection of laboratory and animal colony workers with H. saimiri. Finally, an epithelial cell line from a spontaneous rhesus monkey carcinoma was established. A series of collaborative studies was undertaken in order to characterize this cell line.

Viral Resources Laboratory: In 1977, VRL commitments entailed the production, purification, and characterization of 300 liters per week of retrovirus material for distribution by OPRL. This material consisted essentially of four agents - mouse mammary tumor virus (MMTV), Rauscher murine leukemia virus (RLV), Gross murine leukemia virus (GLV), and baboon leukemia virus (M7). In addition, VRL was charged with the processing of 300 liters per week of types B, C and D retroviruses for the Director of Viral Oncology, FCRC; this material encompassed over 50 different viruses.

In 1977 (Feb. 1977 - Jan. 1978), 37,438 liters of retrovirus material were produced within VRL. This total, 20% above the contractual commitment, was necessitated by increased OPRL needs for virus material. Included in this volume were 10,414 liters (28%) MMTV, 6,422 liters (17%) M7, 6,103 liters (15%) RLV, 1,326 liters (4%) equine infectious anemia virus (EIAV), 1,171 liters (3%) bovine leukemia virus (BLV), 877 liters (2%) GLV, and 11,125 liters (30%) of more than 50 other agents. Thus, VRL increased the previous year's production by 35%, while at the same time vastly expanding the number of viruses produced.

The distribution pattern of virus released by VRL during 1977 indicates that although FCRC/VO investigators received a similar amount of material as in 1976, the percentage that this reflects of the total material distributed was somewhat less (36% vs 43%) than the previous year. Total concentrate produced during the year was 39,613 ml; at year's end 2,298 ml remained in the VRL Virus Repository.

Electron Microscopy Support: The Electron Microscopy Group develops and applies the techniques of scanning (SEM) and transmission (TEM) electron microscopy to meet the needs of investigators in the Viral Oncology Program on both a collaborative and support basis. Although classical techniques for ultrastructural morphologic studies of viruses and cells are still frequently required, recent emphasis has been placed on the molecular aspects of electron microscopy (EM), including immunoelectron microscopy (IEM) and nucleic acid analyses. Novel approaches to quantitative EM, including the use of energy-dispersive X-ray analysis, to augment the visual data obtained in the SEM are being developed. New immunolatex spheres have been developed for surface labeling in the electron microscope. The Kleinschmidt technique for the study of viral nucleic acids in the electron microscope is being applied to studies of retrovirus nucleic acids.

The specific objectives of the Electron Microscopy Group were to (a) develop markers and techniques for the localization of viral proteins in the electron microscope (support of Immunochemistry Laboratory); (b) perform studies on the size, structure, and relatedness of retrovirus genomes (support of Molecular Biology Oncornaviruses Section); (c) study the ultrastructure and morphogenesis of a new viral isolate squirrel monkey retrovirus and equine infectious anemia virus (support of the Biology of Type B Virus Section and the Oncornavirus Immunobiology Section); (d) provide quality control service to the Viral Resources Laboratory which includes performance of particle counts and thin section evaluation of cell lines used in virus production; (e) localize the feline oncornavirus cell membrane antigen by IEM using selected cat sera (support of the Oncornavirus Immunobiology Section); (f) study the localization and expression of various viral proteins (support of the Immunochemistry Section); and (g) determine the effects of amino acid analogs on virus production by EM (support of the Biology of Type C Virus Section).

Quality Control Support: The primary objective of the Quality Control Group is the provision of routine test services for the isolation and identification of mycoplasma from various sources including cell culture and serum.

Currently, the following procedures are in use for routine mycoplasma testing. Cell culture specimens are inoculated into agar medium (1) and the outgrowth of mycoplasma colonies is detected by microscopic observation. For identification, unfixed mycoplasma colonies are reacted directly with fluorescein-conjugated globulin and are examined by use of incident UV excitation (2). Additionally, indicator cell cultures are used to detect mycoplasma contaminants in cells. Test specimens are inoculated into 3T6 cells growing on coverslips. After incubation, one of the two coverslips is stained with a fluorescent antibody conjugate directed against M. hyorhinis (3). The second coverslip is stained with a fluorescent chromatin stain. Such cytochemical techniques have recently been recommended for general usage in mycoplasma detection (4). All mycoplasmas which have been reported as contaminants in cell cultures can be detected by this combination of procedures.

The use of a prescribed cell culture system for research is predicted on the absence of unprescribed organisms. The rationale of the Quality Control Group is to develop and utilize the techniques required to support the Viral Oncology Program in mycoplasma control.

A total of 2,828 tests were performed during this report period.

Those areas of Contractor operations that were partially funded by Viral Oncology, DCCP, NCI are the following:

Environmental Control: Formal inspections of laboratory areas were scheduled and carried out with reports of deficiencies being sent to either the Operations Directorate for correction of engineering and maintenance items or to the concerned scientists for correction of procedural items.

Five accidents involving a potential exposure to biological materials were reported and investigated during the year. Two accidents involved oncogenic viruses, one involved mycoplasma, one Staphylococcus aureus and one potential exposure was with Newcastle Disease Virus (NDV). The accident with NDV involved possible contact with the eyes and a mild conjunctivities may have resulted from this exposure. All accidents occurred during the conduct of standard laboratory procedures.

The FCRC Nuclear Regulatory Byproducts Material license was renewed for a second five year period. The new license changes and in most instances increased the total activity of radioisotopes that can be handled at the FCRC. There are now 38 active radiological programs at the FCRC, 28 of which are radioisotope programs.

A great deal of effort on the part of several individuals in Environmental Control was devoted towards making the P4 recombinant DNA facility ready for certification during 1977. The facility was certified as meeting the HEW, NIH Guidelines in December 1977.

Various training programs in biological, radiological and industrial safety were offered during the year. Two new training programs have been established. One training program was for forklift operators and the second program for all personnel who wear of may wear respirators in the performance of their duties.

Formal inspections of all laboratories will be continued. Each area will be inspected once, twice or three times during the year depending upon potential hazards present and conditions observed during the first inspection.

VO supported approximately 35 percent of this effort.

Animal Breeding and Animal Health Diagnostic Programs: During the period of August 1, 1976 to July 31, 1977, the Animal Production Area of the Frederick Cancer Research Center (FCRC) produced and distributed 621,860 experimental animals including mice, rats and guinea pigs. During the period of May-July, 1977, the Animal Health Diagnostic Section detected mouse hepatitis virus infection in animals housed in both the barrier- and conventional-maintenance production buildings. In August, 1977, Salmonella typhimurium was detected as a low level enzootic infection in the mouse, rat and guinea pig colonies. These outbreaks of infection as well as periodic incidences of Pseudomonas sp. infection in mice produced in conventional-maintenance buildings signaled the fact that the animals being produced were not of sufficient quality for sophisticated cancer research. In August, 1977, Dr. M.G. Hanna, Jr., Director, Cancer Biology became acting chief of the Animal Resources Program and began the reorganization of both the Animal Production Area and the Animal Health Diagnostic Section. Therefore, this report will only cover the six-month period beginning August, 1977, which is the period of total conceptual, as well as technological, reorganization of the Animal Resources Program of the FCRC.

Reorganization efforts have been directed at rectifying: (a) the deficient facilities for maintenance of production of gnotobiotic mice, rats and guinea pigs in the Animal Production Area, (b) inadequate procedures for quarantining incoming animals and poor housekeeping in the quarantine area of the Animal Health Diagnostic Section, and (c) inadequate diagnostic and testing procedures for bacteriological, microbiological and parasitological monitoring of holding, production and experimental rodents.

Because of the importance of this resource to both the Contractor, Litton Bionetics, Inc. (LBI) and the National Cancer Institute (NCI), recommendations were solicited from knowledgeable personnel from both organizations, and an effective joint effort was launched.

The first undertaking was to reestablish the Animal Health Diagnostic Section. Recruitment of key personnel and reorganization of the laboratories was carried out during August and September, 1977, with a great deal of participation by LBI and NCI personnel such as Dr. K. Sibinovic (LBI) and Drs. C. Montgomery and E.A. New (NCI). A new head of the laboratory operation, Ms. S. Conrath, and new head of the quarantine operation, Ms. J. Snow, were appointed. Also, five laboratory technicians and two animal caretakers were added to the staff of the Diagnostic Laboratory.

The next step was to eliminate the infected colonies and reestablish the Animal Production Area, following the successful recruitment of a new manager, Dr. T.W. Davis. At this point a committee was created consisting of both LBI and NCI personnel committed to the total rederivation of the Animal

Health Diagnostic Section as an international reference center for health assessment and care of research animals.

VO supported approximately 25 percent of this effort.

Significance to Biomedical Research and the Program of the Institute: Newly organized programs that were envisioned in January of 1976 were almost completely, if not entirely, implemented during the year 1977. Significant experimental results from a variety of investigations on host-virus interactions, on potential interrelationships among viruses and cell transformational events, on biological, biochemical, and serological characteristics of oncogenic viruses and their components, and on vaccine control have been obtained. This has resulted in new pertinent basic and applied information, providing new insights into the elucidation of the relationships of viruses to neoplastic diseases, to their cause, detection, prevention and control. In addition, increased quantities of high quality viruses and viral reagents, much needed both within FCRC and for outside investigators in support of work in virology, immunology, molecular biology and genetics were made available. The unfortunate spread of contamination throughout the animal breeding area is now under control and the number of important management and physical changes in that and related areas gives considerable assurance against similar future disturbances, and that the quality of laboratory animals should ultimately become vastly improved. Also, work supported by Viral Oncology in the areas of biohazards and environmental control continued to provide increasingly important information to FCRC and outside laboratories on the handling of potentially hazardous biological and chemical materials.

Proposed Course: The currently designed FCRC/VO Program inherently provides a strong but flexible scientific mechanism for providing the continued opportunity to enhance program productivity, by maximizing results of previous efforts as a basis for further work, for rapidly capitalizing on new scientific and technological advances, and for seizing new initiatives in previously untried areas of investigation as related to human cancer.

Plans will remain in effect to continue basic investigations studying the natural history of oncogenic viruses using immunological, virological and molecular biologic methodology. Utilizing specific characterized viral products, e.g., viral envelope proteins and viral induced cell surface antigens, attempts will be made to interrupt viral, chemical and spontaneous neoplasia in appropriate models. Products of tumor cell responses will be completely characterized and assayed for biologic activity. More specifically it is planned that work will proceed along the following lines: (a) Immunochemistry, for the isolation, characterization and antiserum production of important viral or viral-associated proteins; (b) Molecular Biology, to prepare specific transcripts of RNA and DNA oncogenic viruses for studies of virus interrelationships, relationship of viruses to host genomes, transcription of viral genomes under experimental conditions, and preparation and analysis of transcripts specific for certain viral function; (c) Viral and Cell Biology, to develop assays for test viruses, optimal cell lines for virus expression and production, mechanism of virus induction, localization

of viral genomes to specific chromosomes using somatic cell hybridization; (d) Primate (including man) herpesviruses, to study transformation by these viruses or their infectious DNAs, to compare biological activities among various strains, to analyze immunological factors that are conducive or deleterious to cell transformation and tumorigenesis, and (e) Virus, Reagent, and Cell Production to provide a large-scale central resource for working materials.

Finally, fruitful collaboration will continue with on-site NCI investigators engaged in ongoing studies on genetic and vaccine control of virus expression, the carcinogenic and/or co-carcinogenic role of herpesviruses in cell transformation and tumorigenesis, cellular enzymes involved in processes repressing or derepressing cell components, cell membrane property changes induced by virus infection, and the influence of host physiology on oncogenesis and cellular control.

Date Contract Initiated: September 26, 1977

C. 3. PROGRAM MANAGEMENT

b. Cancer Research Emphasis Grants (CREG)

The Virus Cancer Program funded its first Cancer Research Emphasis Grants furing FY 1976. Two announcements asking for proposals were published in the NIH Guide on June 1, 1975. The titles were "Replication of RNA Tumor Viruses" and "Genetics of RNA Tumor Viruses." Thirty-nine proposals were received in response to the announcements and reviewed by Study Sections in January, 1976. Of these proposals, 13 were disapproved and 3 were deferred for site visits or further information; the rest were approved. Ten of the approved applications were funded, in order of priority, during FY 1976. Two additional applications were funded in FY 1977. A total of approximately $0.8 million was obligated during FY 1978 as a result of the first two announcements.

The following grants from these announcements were funded during FY 1978:

CA19341 Haseltine, Sidney Farber Cancer Center
"Replication of RNA Tumor Viruses"

CA19497 Kaji, University of Pennsylvania
"Studies on the Replication of RNA Tumor Viruses"

CA19714 Somers, Eastern Virginia Medical Authority
"Genetics of Murine Sarcoma Virus"

CA19723 Wong, University of Illinois
"Genetics of Helper-Independent Mouse Sarcoma Virus"

CA19725 Vogt, University of Southern California
"Genetics of RNA Tumor Viruses"

CA19729 Watson, University of Montana
"Mechanism of Viral RNA-Directed Polymerization"

CA19873 Lilly, Albert Einstein School of Medicine
"Genetic Control of Resistance to Friend Virus Disease"

CA19931 Lilly, Albert Einstein School of Medicine
"Mechanism of the H-2 Effect on Viral Leukemogenesis"

CA19996 Green, St. Louis University
"Replication of RNA Tumor Viruses"

CA20012 Kang, University of Texas
"Infectious Reticuloendotheliosis DNA Provirus"

On April 1, 1976, two further CREG announcements from the Virus Cancer Program appeared in the NIH Guide. The titles of these were "Malignancy Induced by Small DNA Viruses (Adenoviruses or Papovaviruses)" and "Herpesvirus-Induced Malignancy." In response to the announcements, 44 proposals were received and were reviewed by Study Sections in February and March, 1977. Of these proposals, 10 were disapproved and 34 approved. Twelve of these were funded by FY 1977 and in 1978. The following grants were funded, for a total of approximately $0.7 million, during this fiscal year:

CA21768 Kasamatsu, University of California
"Polypeptides and the Protein-DNA Complex in SV40 Virion"

CA21771 Courtney, University of Tennessee
"Studies of Purified Herpes Simplex Virus Glycoproteins"

CA21772 Lang, Duke University
"Leukemia and Cytomegaloviruses: Reciprocal Effects"

CA21776 Spear, University of Chicago
"Herpesvirus Gene Expression in Transformed Cells"

CA21788 Levine, Princeton University
"Cell Surface Antigens of Viral Transformed Cells and Teratomas"

CA21797 Gurney, University of Utah
"Growth Control and Viral Gene Expression"

CA21799 St. Jeor, Pennsylvania State University
"Herpesvirus-Induced Malignancy"

CA21801 Cabral, Medical College of Virginia
"Herpesvirus Antigens in Transformed Cells"

CA21807 Zimmer, University of Kentucky
"Early Gene Transcription in Adenovirus 2-Infected Cells"

CA21808 Folk, University of Michigan
"Analysis of the Polyoma Genome in Transformed Cells"

CA21824 Green, St. Louis University
"Transforming Proteins of Three Human Adenovirus Groups"

CA21896 Eckhart, Salk Institute
"Transcription in Polyoma-Transformed Cells"